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In re Application of:

Hill and Hannan

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For: GENETIC SEQUENCES ENCODING STEROID AND JUVENILE HORMONE RECEPTOR POLYPEPTIDES AND INSECTICIDAL MODALITIES THEREFOR

CERTIFICATE OF MAILING

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18 Jan 2000
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Tina R. Wilson

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Washington, DC 20231

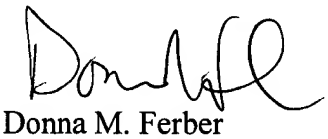
Sir:

Included herewith is one certified copy of the priority document for the above-referenced application. It is as follows:

Australian No. PP 1356, filed 15 January 1998

It is believed that the present submission does not require either a petition for extension of time or the payment of any fee under 37 C.F.R. 1.16-1.17. If this is incorrect, please charge any necessary fee and any extensions of time required to Deposit Account No. 07-1969.

Respectfully submitted,



Donna M. Ferber
Reg. No. 33,878

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone: (303) 499-8080
Facsimile: (303) 499-8089
E-mail: winner@greenwin.com

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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PP 1356 for a patent by COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION filed on 15 January 1998.

WITNESS my hand this Seventh
day of July 1999

KIM MARSHALL
MANAGER EXAMINATION SUPPORT AND SALES

Our Ref: 672846

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Regulation 3:2

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s): Commonwealth Scientific and Industrial Research Organisation
Limestone Avenue
Campbell ACT 2601
AUSTRALIA

Address for Service: DAVIES COLLISON CAVE
Patent & Trade Mark Attorneys
Level 10, 10 Barrack Street
SYDNEY NSW 2000

Invention Title: **Insecticidal modalities.**

The invention is described in the following statement:

INSECTICIDAL MODALITIES

This invention generally relates to insecticidal modalities. More specifically, the invention is concerned with various aspects including screening systems and methods of the identification of insecticidally active agents, methods of the production of biologically active molecules, insect steroid receptors and nucleotide sequences encoding the same, and uses of such receptors and nucleic acid sequences in the regulation of gene expression. This invention is also concerned with partner proteins which associate with insect steroid receptors so as to confer enhanced affinity for insect steroid response elements or enhanced affinity for insect steroids or analogues thereof, or ligands which bind to insect steroid receptors and act as insecticides, or alternatively mimic or potentiate the activity of insect steroids. The invention further extends to compounds which bind to insect steroid receptors and act as insecticidal agents.

International Patent Application No WO91/13167 (applicant, The Board of Trustees of Leyland Stanford University, and hereinafter referred to as WO91/13167) describes the identification, characterization, expression and uses of insect steroid receptors and DNA sequences and protein products, and is incorporated herein in its entirety by reference.

WO91/13167 is concerned with the steroid receptor of the common fruit fly (*Drosophila melanogaster*) which has been found by the present inventors to be temperature sensitive, showing reduced activity at mammalian physiological temperatures above 30°C (such as 37°C), particularly at low concentrations of receptor.

It has been found that by the inventors that it is not possible to use DNA sequences encoding insect steroid receptors from *Drosophila melanogaster* to isolate insect steroid receptors from organisms such as the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, moth, ant, helminth or protozoan. Utilizing a novel screening protocol involving degenerate oligonucleotides from the DNA binding domain of the ecdysone receptors from *Drosophila melanogaster* and *Chironomus tentans* the inventors have solved such problems, therefore allowing the development of the various aspects of the invention hereafter described.

It is noted that the various aspects of the invention hereinafter described enable and/or provide for the identification/production of insecticidally active agents, as well as methods for the regulated production of bioactive molecules.

Ligands which bind to insect steroid receptors and act as agonists or antagonists of insect steroid hormones function as highly specific insecticides, offering significant commercial and environmental benefits.

In accordance with a first aspect of this invention, there is provided a screening system for insecticidally active agents comprising a nucleotide sequence encoding an insect steroid receptor or a fragment thereof, and a nucleotide sequence encoding a partner protein or a fragment thereof which associates with the receptor so as to confer enhanced affinity for insect steroid response elements, enhanced affinity for insect steroids or analogues thereof, or insecticidally active agents and/or thermostability or enhanced thermostability of said receptor, which receptor and partner protein is capable of binding to a candidate insecticidally active agent to form an activated complex, and a nucleic acid sequence encoding a bioactive molecule or a reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex regulates transcription of the nucleic acid sequence, wherein on exposure to said agent expression of the bioactive molecule or reporter molecule correlates with insecticidal activity.

In another aspect of this invention, there is provided a method for the regulated production of a bioactive molecule or a reporter molecule in a cell, said method comprising the steps of introducing into said cell:

a) a nucleotide sequence encoding an insect steroid receptor or a fragment thereof which is capable of binding an insect steroid or analogue thereof, to form an activated complex; and

b) a nucleic acid sequence encoding said bioactive molecule or reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex regulates transcription of the nucleic acid sequence encoding said bioactive molecule or reporter molecule,

wherein exposing the cell to an insect steroid or analogue thereof regulates expression of the bioactive molecule or reporter molecule.

In another aspect this invention relates to a method or assay for screening insecticidally active compounds which comprises reacting a candidate insecticidal compound with an insect steroid receptor polypeptide or fragment thereof encompassing the ligand binding domain, or complex thereof with a partner protein (for example USP as set out in SEQ ID No: 6) or a fragment thereof which encompasses the ligand binding domain, and detecting binding or absence of binding of said compound so as to determine insecticidal activity.

In another aspect the invention relates to synthetic compounds derived from the three dimensional structure of insect steroid receptors which compounds bind to said receptors and have the effect of either inactivating the receptors of potentiating the activity of the receptor.

In another aspect the invention relates to a method for the determination/production of insecticidally active agents which comprises the steps of:

a) expression and purification of an insect steroid receptor or a fragment encompassing the ligand binding domain thereof optionally in association with a partner protein or ligand binding domain thereof, optionally in association with an insect steroid or analogue thereof so as to form a complex;

b) determining the three dimensional crystal structure of said complex and determining thereafter the three dimensional structure of the ligand binding domain; and

c) synthesising compounds which bind to or associate with the ligand binding domain.

In still another aspect the invention relates to an isolated recombinant nucleic acid sequence encoding an insect steroid receptor or a fragment thereof capable of binding an insect steroid, an analogue thereof, or an insecticidally active agent.

In yet another aspect the invention relates to a polypeptide comprising an insect steroid receptor or fragment thereof, which polypeptide is substantially free of naturally associated insect cell components.

In another aspect of this invention there is provided a cell which expresses an insect steroid receptor polypeptide or a fragment thereof which receptor is capable of binding to an insect steroid or analogue thereof or a candidate insecticidally active agent to form an activated complex, and a nucleic acid sequence encoding a bioactive molecule or a reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex promotes transcription of the nucleic acid sequence, wherein said cell on exposure to insect steroid or an analogue thereof, regulates expression of said bioactive molecule or allows detection of said reporter molecule.

In a further aspect of this invention, there is provided an animal (such as a mammal), microorganism, plant or aquatic organism, containing one or more cells as mentioned above. Reference to plants, microorganisms and aquatic organisms includes any such organisms. In

this embodiment of the aspect of the invention, it is to be appreciated that administration of an insect steroid or an analogue thereof to an organism will induce expression of the desired bioactive molecule, such as a polypeptide, with attendant advantages. For example, an induced protein may have a therapeutic effect ameliorating a disease state or preventing susceptibility to disease or may modify in some way the phenotype of an organism to produce a desired effect. In humans, for example, cell transplants (such as liver cells) may under the action of insect steroids, produce desirable hormones such as insulin, growth hormone, growth factors and the like.

The mammal may include, for example, a human, sheep, goat, horse, dog, cow, cat, mouse, rat, rabbit, pig or other mammal. The mammal may be a transgenic mammal.

The screening system may comprise a prokaryotic or eukaryotic cell (such as plant, microorganism, aquatic organism, or animal cell, preferably a mammalian cell), a cell lysate or an aqueous solution.

In this aspect, the "cell" may refer to a single cell, more than one cell such as a clonal group of cells or a heterogenous mixture of cell types which may be prokaryotic or eukaryotic. The cell may form part of an organ (such as a pancreatic cell) or a transgenic animal, plant, microorganism or aquatic organism. Alternatively, the regulatory system may comprise a cell lysate or aqueous solution. In certain embodiments the nucleic acid sequence may be attached to a solid phase matrix.

The insect steroid receptor herein described may be a thermostable insect steroid receptor which does not exhibit reduced activity at plant and animal physiological temperatures above about 30°C.

In the above embodiments, the insect steroid response element or a plurality of such elements may be operably linked to a promoter and optionally one or more enhancer elements as are well known in the art. Response elements generally operate to make transcription responsive to the presence of insect steroid bound to the insect receptor (which may act as a transcription factor). One or more insect steroid response elements may be located within a promoter, and may replace sequences within a selected promoter which confer responsiveness to hormones or other agents which regulate promoter activity. Where response elements are different they may lead to preferential binding of different insect steroids or analogues thereof such that a promoter may be differentially regulated.

The term "cell" as used herein refers to a prokaryotic or eukaryotic cell (such as a plant, microorganism, aquatic organism (such as fish or other marine organism) or animal cell).

It is to be understood that a "fragment" of a nucleotide sequence encoding an insect steroid receptor or partner protein refers to a nucleotide sequence encoding a part or fragment of such a receptor which is capable of binding or associating with an insect steroid or an analogue thereof, or a candidate insecticidally active compound. Fragments would generally comprise in excess of twenty nucleotides and may encode one or more domains of a thermostable insect steroid receptor. For convenience, reference to a nucleotide sequence encoding an insect steroid receptor or a partner protein is to be taken to include a fragment thereof, the encoded protein product of which is capable of binding an insect steroid or an analogue thereof.

Promoters may be cell, tissue, organ or system specific, or may be non-specific. Using specific promoters, production of a bioactive agent may be targetted to a desired cellular site. For example, in transgenic animals such as sheep, it can be envisaged that cells of the transgenic animal may contain a gene encoding an insect steroid receptor, preferably an insect steroid receptor linked to an epidermal specific promoter and a separate gene encoding, for example, epidermal growth factor (EGF) which is functionally linked to one or more insect hormone response elements and may or may not also be linked to epidermal specific promoter elements. On administration of the appropriate insect steroid hormone to the transgenic animal, the activated complex between the insect steroid receptor and insect steroid may bind to the one or more insect steroid hormone response element thereby inducing EGF production solely in epidermal cells which may give rise to defleecing. It is to be understood that this aspect of the invention is independent of the degree of thermostability of the insect steroid receptor The same principal applies to expression of any bioactive molecule or reporter molecule in a specific cell type which is regulated by a transactivating complex between an insect steroid receptor complex and an appropriate insect steroid.

Natural or synthetic DNA fragments (or nucleotide sequences) coding for an insect steroid receptor or fragments thereof, or a partner protein or a fragment thereof, and a bioactive molecule or a reporter molecule linked to one or more insect steroid response element may be incorporated into DNA constructs (expression vectors) capable of introduction into, and expression in, an *in vitro* cell culture, or for introduction into, with or without integration into the genome of a cultured cell, cell line and/or transgenic animal. DNA constructs such as expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA

splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferably, the enhancers or promoters may, for example, be those naturally associated with genes encoding the steroid receptors, although it will be understood that in many cases others will be equally or more appropriate. Examples of other expression control sequences are enhancers or promoters derived from viruses, such as SV40, Adenovirus, Bovine Papilloma Virus, and the like. Similarly, preferred promoters are those found naturally in immunoglobulin-producing cells (see, United States Patent No 4,663,281, which is incorporated herein by reference), but SV40, polyoma virus cytomegalovirus (human or murine) and the LTR from various retroviruses (such as murine leukemia virus, murine or Rous sarcoma virus and HIV) are also available. See, *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, New York, 1983, which is incorporated herein by reference.

Cells may be co-transformed with both a regulatable construct (comprising a nucleic acid sequence encoding a bioactive molecule or reporter operably linked to one or more insect steroid response elements, and optionally operably linked to a nucleic acid sequence encoding a partner protein) and another nucleotide segment encoding an insect steroid receptor. In this aspect, the insect steroid or analogues thereof capable of binding to a thermostable insect steroid receptor will be provided or withheld as appropriate for desired expression of the bioactive molecule.

Non-insect cells are generally insensitive to insect steroids or analogues thereof (for convenience, hereafter reference to an insect steroid will be understood to include reference to an analogue thereof). Thus, exposure of such cells to insect steroids will typically have negligible physiological or other effects on the cells, or on a whole organism. Therefore, cells can grow and express a desired bioactive molecule, substantially unaffected by the presence of the insect steroid itself. The insect steroid will function to cause response either in a positive or negative aspect. For example, it is often desirable to grow cells to high density before expression. In a positive induction system, the inducing insect steroid would be added upon reaching high cell density. As the insect steroid has negligible physiological or other effect on the cells, the only physiological imbalances which result from the expression of the bioactive product itself. In a negative repression system, the insect steroid is supplied until the cells reach a high density. On reaching a high density, the insect steroid may be removed. Introduction of these cells into a whole organism, for example, an animal, would provide the products of expression to that organism.

Nucleotide sequences containing the DNA segments of interest (for example, the insect steroid receptor gene, the recombinant steroid response elements, or both) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example,

calcium chloride transfection is commonly utilized for prokaryotic cells, whereas lipofection or calcium phosphate treatment are often used for other cellular hosts. See, generally, Sambrook *et al*, (1980), *Molecular Cloning: A Laboratory Manual* (2nd Edition), Cold spring Harbor Press; Ausubel *et al*, (1992), *Current Protocols in Molecular Biology*, Greene/Wiley, New York; and Potrykus (1990) *Gene Transfer to Cereals: An Assessment*, *Bio/Technology*, 8:535-542, each of which is incorporated herein by reference. Other transformation techniques include electroporation, DEAE-dextran, microprojectile bombardment, lipofection, microinjection, and others. The term "transformed cell" is meant to also include the progeny of a transformed cell.

In accordance with a further aspect of this invention, there is provided isolated recombinant nucleic acids which upon expression, are capable of encoding an insect steroid receptor or a fragment thereof capable of binding an insect steroid.

The insect steroid receptor as referred to herein is an insect ecdysteroid receptor (hereafter "EcR") capable of binding and forming an active complex with an insect steroid, preferably an ecdysteroid as are well known in the art such as ecdysone (which may hereinafter be referred to as "Ec") or ponasterone A (which may hereinafter be referred to as "PNA"), or analogues thereof.

Nucleotide sequences, according to an aspect of the invention, may encode the ecdysteroid receptor from organisms selected from the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, ant, helminth or protozoan.

SEQ ID NO: 1 shows the cDNA sequence encoding *Lucilia* ecdysone receptor and SEQ ID NO: 2 shows both the cDNA and encoded protein sequence. SEQ ID NO: 3 shows a CDNA sequence the *Lucilia* partner protein (USP) and SEQ ID NO: 4 the encoded protein sequence. SEQ ID NO: 5 shows a cDNA sequence encoding a part of the aphid edcysone receptor and SEQ ID NO: 6 the encoded protein sequence.

The ecdysone receptor gene has been shown to be a member of the steroid and thyroid hormone receptor gene superfamily, a group of ligand-responsive transcription factors. See, Evans (1988) *Science* 240:889-895 which is incorporated herein by reference. These receptors show extensive sequence similarity, especially in their "zinc finger" DNA-binding domains, and also in a ligand (or hormone or steroid) binding domain. Modulation of gene expression apparently occurs in response to binding of a receptor to specific control, or regulatory, DNA elements. The steroid

receptor superfamily is a class of receptors which exhibit similar structural and functional features.

Members of the insect steroid receptor superfamily are characterized by functional ligand-binding (in the present case which binds insect steroid/analogue and candidate insecticidal compounds, which may for convenience be collectively referred to as ligands) and DNA binding domains, both of which interact to effect a change in the regulatory state of a gene operably linked to the DNA-binding site of the receptor. Thus, the receptors of the insect steroid receptor superfamily seem to be ligand-responsive transcription factors. The receptors of the present invention exhibit at least a hormone-binding domain characterized by sequence homology to particular regions, designated E1, E2 and E3.

The members of the insect steroid receptor superfamily are typically characterized by structural homology of particular domains, as defined initially in the oestrogen receptor. Specifically, a DNA binding domain, C, and a ligand-binding domain, E, are separated and flanked by additional domains as identified by Krust *et al* (1986) *EMBO J.*, 5:891-897, which is incorporated herein by reference.

The C domain, or zinc-finger DNA-binding domain, is usually hydrophilic, having high cysteine, lysine and arginine content - a sequence suitable for the required tight DNA binding. The E domain is usually hydrophobic and further characterized as regions E1, E2 and E3. The ligand-binding domains of the present invention are typically characterized by having significant homology in sequence and structure to these three regions. Amino proximal to the C domain is a region initially defined as separate A and B domains. Region D separates the more conserved domains C and E. Region D typically has a hydrophilic region whose predicted secondary structure is rich in turns and coils. The F region is carboxy promixal to the E region (see, Krust *et al*, *supra*). The ligand-binding domain of the members of the insect steroid receptor superfamily is typically carboxyl-proximal, relative to a DNA-binding domain described below. See, Evans (1988) *Science*, 240:889-895. The entire hormone-binding domain is typically between about 200 and 250 amino acids but is potentially shorter. This domain has the subregions of high homology, designated the E1, E2 and E3 regions - which may be collectively referred to as the "E region". Fragments of insect steroid receptors and partner proteins capable of binding insect steroids, and candidate insecticidally active compounds comprise an E-region or a sufficient portion of the E-region to allow binding.

The present invention provides for the isolation of ecdysteroid binding receptors from various organisms of the class *Insecta*, such as the Australian sheep blowfly (*Lucilia*), common housefly, sandfly, aphid, scale insect, leaf hopper, beetle or protozoan.

Where reference is made to thermostability of insect steroid hormone receptors, this generally refers to the capacity of such receptors to activate genes linked to insect steroid hormone response elements, which when ligated to DNA encoding a bioactive molecule, results in regulation of transcription of said bioactive molecule.

Reference to "insect steroid hormone response elements" generally refers to one or more ecdysteroid response elements such as ecdysone response element hsp27 (EcRE) or any other nucleotide sequence capable of binding ecdysteroid receptors (such as associated with E75, E74 or other *Drosophila* early genes), which are well known in the art (Riddihough and Pelham, 1987, incorporated herein by reference).

Another aspect of this invention relates to a recombinant nucleic acid comprising one or more insect steroid response element capable of binding to an insect steroid receptor operably linked to a promoter sequence which in tum is operably linked to a DNA sequence encoding a bioactive molecule (such as a protein, peptide or RNA). The insect hormone receptor response element may comprise multiple repeats of response elements capable of interacting with insect steroid receptors.

The invention also relates to a DNA sequence encoding an insect steroid receptor or a fragment thereof encompassing the ligand binding domain, and a partner protein or a fragment thereof encompassing the ligand binding domain thereof. Such a DNA sequence may be used to express the encoded protein product for use in screening assays for identifying insecticidally active compounds, or for three dimensional structure analysis.

Another aspect of this invention mentioned above is concerned with a polypeptide comprising an insect steroid receptor or fragment thereof. The polypeptide may be substantially free of naturally associated insect cell components, or may be in combination with a partner protein which associates with the insect steroid receptor so as to confer enhanced affinity for insect steroid response elements, enhanced affinity for insect steroids or analogues thereof.

In another aspect this invention comprises a partner protein or a fragment thereof. Partner proteins or fragments thereof associate with insect steroid receptors so as to confer enhanced

affinity for insect steroid response elements, enhanced affinity for insect steroids or analogues thereof, or insecticidally active agents and/or thermostability or enhanced thermostability of said receptor. A partner protein may be endogenously produced by a cell, or may be produced by a cell following introduction into the cell of a nucleotide sequence encoding the partner protein. An example of such a protein is the *Lucilia* protein USP, a product of the *Lucilia* homologue of the *Drosophila* protein ultraspiracle (see, for example, Yao *et al* 1993). A DNA sequence encoding the *Lucilia* USP is set out in SEQ ID NO: 3 and the translated protein in SEQ ID NO: 4. Each organism which expresses an ecdysone receptor, also expresses a USP. It is preferred to use the USP or a fragment thereof from the organism in question. However USP sequences from different organisms may be used, to varying effect, as long as they associate with the insect steroid receptor. USP proteins, and nucleotide sequences encoding the same contain the same general domain structure as insect steroid receptors herein described. The so-called E-domain associates with the E-domain of the insect steroid receptor. Reference to "substantially free of naturally associated insect cell components" refers to at least 80% purity, preferably more than 90% purity, and more preferably more than 95% purity. Normally, purity is measured on a polyacrylamide gel with homogeneity determined by staining of protein bonds. Alternatively, high resolution may be necessary using HPLC or similar means. For most purposes, a simple chromatography column or polyacrylamide gel may be used to determine purity.

A protein which is chemically synthesized or synthesized in a cell system different from an insect cell from which it naturally originates would be free of naturally-associated insect cell components.

SEQ ID NO: 2 sets out the amino acid sequence of the *Lucilia* ecdysone receptor which comprises 759 amino acids. The amino acid sequence set out in SEQ ID NO: 2 may be varied by the deletion, substitution or insertion of one or more amino acids. Such variants which are capable of binding insect steroids form part of the present invention.

As previously mentioned, insect steroid receptors comprise a DNA-binding domain, C, and a ligand-binding domain, E, and are separated and flanked by additional domains as identified by Krust *et al*, (1986), *EMBO. J.*, 5:891-897, which is incorporated herein by reference.

Insect steroid receptors or partner proteins, or fragments thereof, may be produced according to techniques known in the art, such as by expression of the protein product in a host cell transformed with nucleic acid encoding the desired protein which is either secreted from the cell or accumulates in the cell. The expressed protein may be purified by standard techniques, such

as column chromatography (using various matrices which interact with the protein products, such as ion exchange matrices, hydrophobic matrices and the like), affinity chromatography utilizing antibodies specific for the protein or other ligands such as dyes or insect steroids which bind to the protein. Alternatively, proteins may be synthesized by standard protein synthetic techniques as are well known in the art.

Insect steroid receptor polypeptides or ligand binding domains, or their complexes with partner proteins or ligand binding domains thereof which confer enhanced affinity for insect steroid response elements are used to develop novel insecticides, or to produce highly active compounds which mimic the activity of insect steroids. Methods are now well established for the three dimensional structural determination of proteins utilizing techniques such as X-ray crystallography and nuclear magnetic resonance analysis. The three dimensional structure of a thermostable insect steroid receptor polypeptide or a ligand binding domain thereof optionally in association with a partner protein or a ligand binding domain thereof, further optionally in association with a ligand (insect steroid or analogue (compounds which mimic the effect of insect steroids) thereof) enables the production of compounds which bind to the ligand binding domain (see, for example, Von Itzstein, (1993) *Nature* Vol 363:418-423; and Bugg *et al*, (1993) *Scientific American*, December Issue, pages 60-66). In this manner, insecticidal compounds may be produced which bind to the ligand binding domain of the receptor. In the same way, compounds may be developed which have a potentiated interaction with the insect steroid receptor over and above that of the physiological insect steroid which binds to the receptor.

In another embodiment of the invention as described above, there is provided a method or assay for screening insecticidally active compounds which comprises reacting a candidate insecticidal compound with an insect steroid polypeptide or complex thereof with a partner protein and detecting binding or absence of binding of said compound so as to determine insecticidal activity. In this aspect, the protein or complex thereof is used in assays to determine whether candidate insecticidal molecules bind to the receptor polypeptide. Those molecules that do represent potential insecticidal compounds. Such methods or assays may, for example, involve binding the insect steroid receptor polypeptide to a support such as a plurality of polymeric pins, whereafter the polypeptide resident on the plurality of pins is brought into contact with candidate insecticidal molecules for screening. The molecules being screened may be isotopically labelled so as to permit ready detection of binding. Alternatively, reporter molecules may be utilized which bind to the insect steroid receptor candidate molecule complex. Alternatively, compounds for screening may be bound to a solid support, such as a plurality of pins which are then reacted with

the thermostable insect steroid receptor or complex with a partner protein. Binding may, for example, be determined again by isotopically labelling of the receptor, or by antibody or other reporting agent.

In vivo assays may be used to screen for insecticidally active compounds, such as those compounds which act as agonists, antagonists or competitors of the binding of insect steroid by ecdysone receptor (*LcEcR*). In such assays, expression plasmids containing *LcEcR* or EcR from insects, or a hybrid EcR may be co-transfected into cells with a plasmid containing an ecdysone response element and a reporter sequence. Addition of potential insecticidal substance, in the presence or absence of insect steroid, induces reporter synthesis for subsequent assay. The effects of a potential insecticidal substance can thus be measured in such a system by assay of reporter.

In addition, substances may be screened for insecticidal activity by assessing their ability to bind to EcR and partner protein (for example, USP as set out in SEQ ID No 6) ligand binding domains *in vitro*. Competition assays involving the native insect steroid may be employed to assess insecticidal activity.

In another aspect this invention relates to synthetic compounds (which may be referred to as ligands) derived from the three dimensional structure of insect steroid receptors which compounds are capable of binding to said receptors which have the effects of either inactivating the receptors (and thus acting as antagonists) or potentiating the activity of the receptor. The compounds may bind strongly or irreversibly to the ligand binding site or another region of the receptor and act as agonists or antagonists of insect steroids. Such compounds would have potent insecticidal activity given the key role of insect steroids in insect physiology and biochemistry. Such compounds would also possess a unique specificity.

This invention will now be described with reference to the following non-limiting examples and figures in which:

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Structure of an EcR encoding plasmid and EcR gene.

FIGURE 2: Structure of the reporter plasmid $p(EcRE)_7CAT$.

FIGURE 3: Hormone dependence of reporter gene expression at 37°C. The CAT activity in CHO cells co-transfected with the indicated plasmids in the presence of 20μM

PNA(+) or absence of hormone (-) and expressed as a ratio over cells transfected with pMMTV-CAT (containing no EcRE) and $pSV40_p$, the expression vector without an inserted EcR gene, 2.5 µg of effector plasmid, and 2.5 µg of reporter plasmid were added to each 6 cm diameter dish.

FIGURE 4: Temperature effect on reporter gene induction by PNA. A constant amount of 2.5 µg reporter plasmid $p(EcRE)_7CAT$ DNA and the amount indicated of receptor expressing plasmid pSVp-EcR DNA were employed to co-transfect CHO cells which were subsequently cultured at 30°C or 37°C.

FIGURE 5: Temperature effect of reporter gene activation by EcR activity as a transcription factor. Co-transfection assays were performed in the absence or hormone with receptor expressing plasmid pSV40-EcR and the reporter plasmid p2EcRE-MMTV-CAT having two copies of an EcRE or a similar reporter plasmid without the EcRE's, pMMTV-CAT. Induction has been calculated relative to a co-transfection with $pSV40_p$ (the expression vector without inserted EcR gene) and pMMTV-CAT (reporter plasmid without inserted EcRE's).

FIGURE 6: *Lucilia* ecdysone receptor (*LcEcR*) function in vivo. As described in Example 1, CHO cells were cotransfected with (1) one of the EcR expression plasmids: pSGDmEcR, pSGLcEcR or the parental expression plasmid pSG5 as a control, at 1ug/ml, (2) $p(EcRE)_5CAT$ (1 ug/ml) a CAT reporter plasmid and (3) an independent reporter, pPGKLacZ, at 1 ug/ml. CAT expression was induced with Muristerone A at either 10µM or 50µM while control cells received only the carrier ethanol. ELISA kits were used to quantify the synthesis of CAT and β-galactosidase in extracts of cells 48 hours after transfection. Variations between experiments were removed by normalising the level of CAT to β-galactosidase in the same extract. Fold induction represents the normalised values for CAT gene expression in cells transfected with pSGDmEcR, pSGLcEcR or pSG5 in the presence of hormone divided by the normalised values for CAT gene expression in cells transfected with the same plasmid but in the absence of hormone. The average values of three independent experiments are shown and the error bars indicate standard error of the mean.

FIGURE 7: pSGLD (that is, LcDm) and pSGDL (that is, DmLc) contain chimeric EcRs produced by domain swapping between DmEcR and *LcEcR*: pSGLD (that is, LcDm) codes for the *LcEcR* from the NH_2-terminus to the end of the DNA Binding Domain, followed by the DmEcR D domain and Hormone Binding Domain to the DmEcR COOH-terminus; pSGDL (that is, DmLc) codes for the DmEcR from the NH_2-terminus to the end of the DNA Binding Domain followed by the *LcEcR* D

domain and Hormone Binding Domain to the *LcEcR* COOH -terminus. Co-transfection assays as in Figure 6 using above described plasmids and CAT-reporter plasmid $p(EcRE)_5$ CAT (1ug/ml) and an independent reporter pPGKLacZ at 1 ug/ml. CAT/b-Gal (%) refers to CAT reporter activity expressed as a percentage relative to β-galactosidase activity produced by the internal control reporter, pPGKLacZ.

FIGURE 8: (a) Histogram of non-specific and total binding activity in Sf21 cells containing LcEcRDEF-USPDEF. X Axis: The non-specific and total binding of H^3-PNA in Sf21 cells containing EcRDEF-USPDEF. Y Axis: the H^3-PNA counts obtained from the experiment.

(b) Histogram of non-specific and total binding activity in Sf21 control cells containing the baculorivus only (not the inserts). Y Axis: the H^3PNA counts obtained from the experiment.

SUMMARY OF SEQUENCE LISTING

SEQ ID No: 1: The cDNA sequence which encodes the *Lucilia* ecdysone receptor.

SEQ ID No: 2: The encoded protein product of the *Lucilia* ecdysone receptor.

SEQ ID No: 3: The cDNA sequence which encodes the *Lucilia* partner protein.

SEQ ID No: 4: The encoded protein product of the ecdysone *Lucilia* partner protein.

SEQ ID No: 5: The cDNA sequence which encodes part of the aphid ecdysone receptor.

SEQ ID No: 6: The encoded protein product of part of the aphid ecdysone receptor.

EXAMPLE 1

Construction of Receptor Expressing Plasmid pSV40-EcR:

The 3110 base pair Fsp1-HindIII fragment, containing the complete 2634 base pair coding region for the *Drosophila melanogaster* ecdysone receptor (EcR), with 214 base pairs of 5′ leader sequence and 258 base pairs from the 3′ untranslated region, was cut out of a plasmid bearing the EcR-cDNA (Koelle *et al*, 1991). The fragment was ligated into the BamH1 site of the expression pSG5 (Greene *et al*, 1988) to give pSV40-EcR (Figure 1).

Construction of Reporter Plasmids:

The reporter plasmid $p(EcRE)_7$-CAT was constructed by insertion of seven copies of the hsp27 ecdysone response element from the hsp27 gene (Riddihough and Pelham, 1987) into the HindIII

site of the plasmid pMMTV-CAT (Hollenberg and Evans, 1988), 93 base pairs upstream of the transcription start site of the MMTV promoter.

The reporter plasmid p(EcRE)$_7$-CAT (Figure 2) was constructed by insertion of seven copies of a 33 base pair sequence containing a central 13 base pair palindromic ecdysone response element (EcRE) into the HindIII site of pMMTV-CAT.

Cell Culture and Transient Transfection:

Chinese hamster ovary (CHO) cells were maintained in 50% (v/v) Dubbecco's modified Eagle's medium (DMEM) and 50% (v/v) Hamm F12 nutrient mixture (GIBCO) supplemented with 10% (v/v) foetal bovine serum. Transfection was carried out by the DNA-calcium phosphate co-precipitation method (Ausbel *et al*, 1992). One day before transfection, CHO cells were plated out at 5 - 8 x 10^5 cells per 6 cm diameter culture dish in the above medium. Three hours before the addition of the DNA-calcium phosphate co-precipitate the cells were washed with phosphate buffered saline (PBS, Sambrook, *et al*, 1989) and cultured in fresh DMEM plus 10% (v/v) foetal bovine serum. The cells were incubated in the presence of the co-precipitate for eighteen hours before excess DNA was washed away with PBS. The cells were then cultured for another day in DMEM/F12 supplemented with 10% (v/v) foetal bovine serum with or without added ponasterone A (PNA) before harvesting. All transfections included, in addition to the EcR expression and reporter plasmids, a β-galactosidase expressing plasmid pPgK-LacZ (McBurney *et al*, 1991) which served as an internal control on transfection efficiency, and pUC18 DNA to bring the total amount of DNA added per dish to 10 μgm. Cells were washed with PBS and harvested by mechanical scraping in 0.25 M Tris-HCl (pH 7.8) and disrupted for enzyme extraction by three freeze-thaw cycles. CAT and β-galactosidase activities were assayed as described in Sambrook *et al*, (1989). CAT activity is shown in Figure 3. Cells transformed with (ECRE)$_7$-CAT and SV40p-EcR clearly showed induction of CAT activity in the presence of PNA.

We have observed that the ecdysone receptor can lead to stimulation of expression from an ecdysone response promoter in cells, for example, CHO, but not in other, for example, CV-1. This presumably reflects a cell type specific distribution of at least one other transcription factor essential for ecdysone responsiveness. Cell free transcription lysates from expressing and non-expressing cell lines can be prepared and the cell type specificity of ecdysone responsiveness can be confirmed in these lysates. By fractionating nuclear proteins from the expressing cell tissues and supplementing the non-expressing lysates with these, the essential auxiliary factors can be defined and the genes encoding them cloned. Co-transfection of the receptor and

auxiliary factor expressing genes could remove limitations imposed by cell type restricted ecdysone responsiveness.

Testing the Effect of Temperature on Transient Expression:

Cells were plated out at 37°C sixteen to twenty hours before transfection. After washing away the DNA, the cells were cultured for two hours in fresh medium with or without hormone and the dishes divided into two sets. One set was cultured for another day at 37°C before harvesting for CAT and β-galactosidase assays. The other set was cultured for three days at 30°C before assaying enzyme activities. Results are shown in Figures 4 and 5. DNA induced CAT activity is clearly decreased at 37°C compared to 30°C (presumably due to EcR instability, that is, thermolability), this being particularly noticeable at low receptor concentration.

EXAMPLE 2

Attempted screening of L. cuprina DNA library with a segment of *D. melanogaster* EcR

A 627 bp Eco - Kpn I fragment encompassing the DNA binding domain from the DmEcR was isolated, radioactively labelled and used to screen a lambda cloned L. cuprina genomic library (prepared by CSIRO, division of Entomology). Twenty-four regions of the plates showing potential positive hybridisation to the D. melanogaster probe were identified. However, second round screening of plaques representative of the 24 starting potential positives failed to yield any plaque giving a reproducible positive signal when hybridised to the *D. melanogaster* probe.

EXAMPLE 3

Cloning and Characterization of Nucleic Acid Encoding the *Lucilia* Ecdysone Receptor

1. A 105 base pair fragment of the DNA binding domain of the *Lucilia* ecdysone receptor (LEcR) was cloned from the *Lucilia* genome by PCR for use as a probe, by using the redundant primers:

   (i) Rdna3 (32mer with EcoRI site)
   5′-CGG-AAT-TCC-GCC-TCT-GGT-TA(C/T)
   -CA(C/T)-TA(C/T)-AA(C/T)-GC 3′

   (ii) Rdna4 (32mer with BamHI site)
   5′-CGC-GGA-TCC-(G/A)CA-CTC-CTG-ACA-CTT-TCG-(C/T)CT-CA 3′

These probes were designed based on the conservative amino acid sequence of the DNA binding domains of DmEcR and CtEcR. Sequence data from two other members of the steroid receptor superfamily of *D. melanogaster*, that is *Drosophila* hormone receptor 3, DHR3 (Koelle, *et al* 1992) and *Drosophila* early gene, E75 (Segraves and Hogness, 1990) was used in the primer designs to minimise cloning the *L. cuprina* homologs of these

proteins. To facilitate cloning, the 5' end of R1 contained in *Eco*RI site and the 5' end of R2 contained a *Bam*HI site.

The DNA fragment and associated primer were then cloned into *p*Bluescript SK + after digestion using the enzymes EcoRI and BamHI and purification of the digest by agarose gel electrophoresis and electroelution of the product band.

The polymerase was Taq DNA polymerase from Promega and the PCR-programme was:

97°C/5 minutes, 50°C hold; add polymerase 50°C/5 minutes;
72°C/3 minutes, 94°C/1 minute, 50°C/1 minute - repeat twice;
72°C/3 minutes, 94°C//1 minute, 55°C/1 minute - repeat forty times;
72°C/10 minutes.

2. For probe preparation the insert was cut out of the vector with EcoR1 and BamHI and ^{32}P labelled using the GIGAprime DNA Labelling Kit (Bresatec Limited, Adelaide, Australia) essentially according to the manufacturer's instructions except that random primers were replaced with the specific primers *Rdna3* and *Rdna4* (see above). Unincorporated label was removed by size exclusion chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used at 10^6 cpm/ml in hybridizations.

3. Independent cDNA libraries were then prepared in *Lambda/ZapII* by random priming and oligo-dT priming respectively, for screening. Both of these libraries are superior to existing *Lucilia* libraries in infectivity per millilitre and contain good insert sizes.

The particulars of the libraries are as follows:

Libraries

Tissue Source:	Late third instar *Lucilia* larvae cDNA.	
Vector:	Lambda ZAPII insertion vector (Stratagene).	
Cloning site:	*Eco*RI	
Priming Methods:	(1)	Oligo-dT primed for first library
	(2)	Random-primed for second library
Titres:	(1)	1.9×10^6 pfu/ml for oligo-dT primed library-Primary
		7.5×10^{10} pfu/ml for oligo-dT primed library-Amplified
	(2)	1.3×10^6 pfu/ml for random-primed library-Primary
		3.4×10^{10} pfu/ml for random-primed library-Amplified

Insert sizes: (1) 0.5 - 4 kbp for oligo-dT primed library

(2) 0.5 - 4 kbp for random-primed library

4. The prepared phage-libraries were then screened by lifting 500,000 plaques from each library in duplicate onto Hybond N membranes (Amersham) and hybridizing under low stringency conditions to the ^{32}P labelled probe produce at point 2 above for twenty four hours at 37°C.

The hybridization solution was as follows:

42% formamide (wv)

5 x SSPE

5 x Denhardt's solution

0.1% sodium dodecyl sulphate (w/v)

The membranes were then washed under low stringency conditions at 37°C with

0.1% sodium dodecyl sulphate (w/v)

2 x SSC

Following washing positive plaques were detected by autoradiography using XOMAT-AR film (Kodak) for two to three days at -70°C.

From the screening two positive plaques were obtained for the random-primed library and one positive plaque obtained for the oligo-dT primed library.

*p*Bluescript phagemids (each containing a cDNA insert) were then excised *in vivo* from positive plaques using the Exassist Helper Phage system (Stratagene).

Finally, sequencing using the USB Sequenase Version 2.5 Kit was carried out to determine that two genuine fragments of *Lucilia* EcR were obtained from the random primed cDNA library. These were of 561 base pairs and 1600 base pairs length respectively. The fragments provide both the important DNA binding domain and the hormone binding domain as well as the entire 3′ end of the derived full length clone.

A full length clone was obtained from the oligo-dT primed cDNA library. The DNA sequence is set forth in SEQ ID NO: 1. The protein coding sequence of 2271 base pairs is contained within a cDNA fragment of approximately 3400 base pairs as is set out in SEQ ID NO: 2.

EXAMPLE 4

Cloning and Characterization of Nucleic Acid Encoding an Aphid Ecdysone Receptor

1. A 105 base pair fragment of the DNA binding domain of a *Myzus persicae* ecdysone receptor (MEcR) was cloned from the *Myzus* genome by PCR for use as a probe, by using the redundant primers:

(i) Rdna3 (32mer with EcoRI site)
5′-CGG-AAT-TCC-GCC-TCT-GGT-TA(C/T)-
CA(C/T)-TA(C/T)-AA(C/T)-GC 3′

(ii) Rdna4 (32mer with BamHI site)
5′-CGC-GGA-TCC-(G/A)CA-CTC-CTG-ACA-CTT-TCG-(C/T)CT-CA 3′

The DNA fragment and associated primer were then cloned into *p*Bluescript SK + after digestion using the enzymes EcoRI and BamHI and purification of the digest by agarose gel electrophoresis and electroelution of the product band.

The polymerase was Taq DNA polymerase from Promega and the PCR-programme was:
97°C/5 minutes, 50°C hold; add polymerase 50°C/5 minutes;
72°C/3 minutes, 94°C/1 minute, 50°C/1 minute - repeat twice;
72°C/3 minutes, 94°C//1 minute, 55°C/1 minute - repeat forty times;
72°C/10 minutes.

2. The sequence of the insert was obtained using the USB Sequenase version 2.0 Kit. On the basis of this sequence two authentic polymerase primers were synthesized:

Mdna1 (23mer)
5′ GCC TCG GGG TAT CAC TAT AAC GC 3′
Mdna2 (23mer)
5′ GCA CTC CTG ACA CTT TCG TCT CA 3′

For *Myzus* probe preparation the *Myzus* genome DNA insert was cut out of the vector with EcoRI and BAMHI and ^{32}P labelled using the GIGAprime DNA Labelling Kit (Bresatec Limited, Adelaide, Australia) essentially according to the manufacturer's instructions except that random primers were replaced with the specific primers *Mdna1* and *Mdna2* (see above). Unincorporated label was removed by size exclusion chromatography over Biogel-P60 (Biorad Ltd, Sydney, Australia). The probe was used at 10^6 cpm/ml in hybridizations.

3. Independent cDNA libraries were then prepared in *Lambda/ZapII* by random priming and oligo-dT priming respectively, for screening. Both of these libraries are superior to existing *Myzus* libraries in infectivity per millilitre and contain good insert sizes.

The particulars of the libraries are as follows:

Libraries

Tissue Source:	Late third instar *Myzus* larvae cDNA.	
Vector:	Lambda ZAPII insertion vector (Stratagene).	
Cloning site:	*Eco*RI	
Priming Methods:	(1)	Oligo-dT primed for first library
	(2)	Random-primed for second library
Titres:	(1)	1.9×10^6 pfu/ml for oligo-dT primed library-Primary
		7.5×10^{10} pfu/ml for oligo-dT primed library-Amplified
	(2)	1.3×10^6 pfu/ml for random-primed library-Primary
		3.4×10^{10} pfu/ml for random-primed library-Amplified
Insert sizes:	(1)	0.5 - 4 kbp for oligo-dT primed library
	(2)	0.5 - 4 kbp for random-primed library

4. The random provided *Myzus* cDNA phage-library was then screened by lifting 500,000 plaques in duplicate onto Hybond N membranes (Amersham) and hybridizing under low stringency conditions to the ^{32}P labelled probe produce at point 2 above for twenty four hours at 37°C.

The hybridization solution was as follows:

42% formamide (w/v)
5 x SSPE
5 x Denhardt's solution
0.1% sodium dodecyl sulphate (w/v)

The membranes were then washed under low stringency conditions at 37°C with 0.1% sodium dodecyl sulphate (w/v) and 2 x SSC.

Following washing a positive plaque was detected by autoradiography using XOMAT-AR film (Kodak) for two to three days at -70°C and has been plaque purified. The purified DNA was sequenced according to standard procedures.

A partial clone was obtained from the random primed aphid cDNA library. DNA sequencing of this clone is recorded in SEQ ID NO: 5. The protein coding sequence of 585 base pairs includes a DNA binding domain (base pair position 137 to 337) and is recorded in SEQ ID NO: 6.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within its spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

EXAMPLE 5

Lucilia Ecdysone Receptor (*LcEcR*) Function *In Vivo*

Plasmid pF3 was constructed in three steps as follows: p5S1 was digested with EarI, end-filled and a 300 bp fragment containing the 3' end of *LcEcR* was subcloned into the HindII site of pUC19 to construct pEAR such that the *LcEcR* 3' end was oriented towards the KpnI site. p5S1 was also digested with either (1) ApoI and PstI to isolate the 5' end of *LcEcR* as a 179 by fragment A, (2) PstI and SpeI to isolate a 1650 bp fragment B and (3) SpeI and BglII to isolate a 203 bp fragment C. pEAR was digested with BglII and KpnI to isolate the 3' end of *LcEcR* as a 313 bp fragment D. DNA fragments A, B, C and D were isolated by agarose electrophoresis and ligated together into pBluescriptSK+ which had been digested with EcoRI and KpnI. The resulting plasmid, pF3, contains the complete coding region of the *LcEcR* encompassed as a 2368 bp fragment between two BamHI sites. pSGLcEcR was constructed by cloning *LcEcR*, as the 2368 bp BamHI fragment from pF3, into the BamHI site of the mammalian expression vector pSG5 (Stratagene). LcK8 is a clone of pSGLcEcR.

pSGDmEcR is the plasmid referred to as pSV40-EcR in Example 1 above where its construction is described.

As described in Example 1, CHO cells were cotransfected with (1) one of the EcR expression plasmids: pSGDmEcR, pSGLcEcR or the parental expression plasmid pSG5 as a control, at 1 ug/ml, (2) p(EcRE)$_5$CAT (1ug/ml) a CAT reporter plasmid and (3) an independent reporter, pPGKLacZ, at 1ug/ml. CAT expression was induced with Muristerone A at either 10uM of 50uM while control cells received only the carrier ethanol. ELISA kits were used to quantify the synthesis of CAT and β-galactosidase in extracts of cells 48 hours after transfection. Variations

between experiments were removed by normalising the level of CAT to B-galactosidase in the same extract. Fold induction represents the normalised values for CAT gene expression in cells transfected with pSGDmEcR, pSGLcEcR or pSG5 in the presence of hormone divided by the normalised values for CAT gene expression in cells transfected with the same plasmid but in the absence of hormone. The average values of three independent experiments are shown and the error bars indicate standard error of the mean.

The *LcEcR* from Example 3 is biologically active *in vivo* as is evident from Figure 6. CAT induction is observed at both 50μm and 10μm steroid (Muristerone A), with about 30 and 15 fold induction respectively.

Potential insecticidal substances acting by interaction with the *EcR* are screened by addition of the compound to the *in vivo* assay. Substances are added in an amount from .05uM to 100uM. Candidate insecticidal compounds are identified.

EXAMPLE 6

Chimeric Ecdysone Receptors

Chimeric ecdysone receptors are produced and designated pSGLD (that is, LcDm) and pSGDL (that is, DmLc). These receptors contain chimeric EcRs via domain swapping between DmEcR and *LcEcR*: pSGLD (that is, LcDm) codes for the *LcEcR* from the NH_2-terminus to the end of the DNA Binding Domain, followed by the DmEcR D domain and Hormone Binding Domain to theDmEcR COOH-terminus; pSGDL (that is, DmLc) codes for the DmEcR from the NH_2-terminus to the end of the DNA Binding Domain followed by the *LcEcR* D domain and Hormone Binding Domain to the *LcEcR* COOH-terminus.

As shown in Figure 7, hybrid receptors between the *LcEcR* DNA binding domain and DmEcR hormone binding domain and vice versa show bioactivity when measured in the CAT assay of Example 5, where expression plasmids are DmEcR (Dm), LcDm, DmLc, LcK8 and pSG5. Significant bioactivity is observed at 50 um hormone (Muristerone A) with the hybrid plasmids, which show a similar extent of bioactivity to that of the DmEcR.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within its spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to

or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

EXAMPLE 7

The cDNA encoding a *Lucilia* partner protein which may be designated LcUSP was isolated using as a DNA probe sequences based on the USP of *Drosophila melanogaster*. The cloning and characterisation was carried out according to Examples 1 and 3. The DNA sequence and encoded protein products are set out in SEQ ID NO: 3 and SEQ ID NO: 4.

EXAMPLE 8

DNA constructs for the expression of ligand binding domains of insect steroid receptors and partner protein are prepared. The protein products which associate on expression, or which may be separately -purified and then associated together may be used in high through-put assays or three dimensional structural analysis.

A Sac I - Hind III fragment encoding most of the ligand binding domain of the *D. melanogaster* ecdysone receptor was cut out of a plasmid bearing the EcR DNA. (Koelle et al. m 1991). The fragment was cloned in to the Sac I - Hind III cleaved expression vector pQE31 to give pQE31DmECR.

A baculovirus for simultaneous expression of the ligand binding domains of *D. melanogaster* EcR and USP ligand binding domains in insect cells is constructed. A EcoR I - Hind III fragment from pQE31DmECR encoding an oligo His tag and most of the linker domain together with all of the hormone binding domain of the *D. melanogaster* EcR was ligated into EcoR I - Hind III cleaved PfastBac DUAL to give a new plasmid pDmEcR.DUAL. A Hind III - Nsi I fragment encoding most of the linker and all of the ligand binding domain of *D. melanogaster* USP was cut out of the plasmid pZ7-1 and ligated into Nco I - Nsi I cleaved pDmEcR.DUAL. A "FLAG" encoding sequence was incorporated upstream of and in phase with, the sequence encoding the linker and ligand binding domain of USP by ligation into the unique Sma I site to give pDmEcR.USP.DUAL. The correct orientation of the FLAG segment was selected by sequencing. The segment of pDmEcR.USP.DUAL encoding the tagged EcR and USP sequences under the control of polyhedrin and p10 promoters, respectively, was recombined into a baculovirus genome employing the Tn7 transposition system (Luckow et al 1993 J. Virol, 67 4566) The polypeptide products were then co-expressed and combined to form a complex.

In a similar manner to that described above, a baculovirus expression vector for the simultaneous expression of the ligand binding domains of *Lucilia* EcR (LeEcR) and *Lucilia* USP (LcUSP) was prepared. The plasmid containing the ligand binding domains of LcEcR and USP encoding the tagged EcR and sequences under the control of polyhedrin and p10 promoters, respectively, was recombined into a baculovirus genome as described above. The polypeptide products were then co-expressed and combined to form a complex.

Expression was examined by immunoblot analysis. Antibodies directed against oligo-His and FLAG tags detected bands on immunoblot analysis of approximately the predicted sizes for the expressed EcR and USP ligand binding domains, respectively, in extracts from insect Sf21 cells infected with the recombinant baculovirus. The protein detected by anti-oligo-His was greatly enriched utilising a nickel-NTA resin (Qiagen) and the FLAG-labelled protein purified on FLAG M2 Affinity Gel (Kodak).

Furthermore, binding assays, carried out by a modification of the method of Yund *et al* (1978), demonstrated a significant increase in the binding of the tritiated ecdysone analogue ponasterone A in cells infected by recombinant virus indicating correct folding and association of the two protein subunits (Figure 8). Cells infected by a control virus displayed neither antibody-positive bands on western analysis nor specific binding of tritiated hormone above background.

EXAMPLE 9

In-vitro Screening for the Detection of Insecticidal Compounds

Insect steroid polypeptides optionally associated with a partner protein produced according to Example 8. are coupled to pins (according to the procedure of Geysen *et al,* (1987) *J. Immunol. Methods* 102, 259-274, incorporated herein by reference) and reacted with candidate insecticidal compounds generally in an amount of from 0.05 µm to 100 µm. Binding of compounds is detected using standard procedures, and compounds having insecticidal activity identified.

In one group of experiments insecticidal compounds specific to *Lucilia* are developed, and in another group of experiments insecticidal compounds specific to aphids are developed.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Commonwealth Scientific and Industrial Research Organisation

(ii) TITLE OF INVENTION: Insect steroid receptors

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:	DAVIES COLLISON CAVE
(B) STREET:	LEVEL 10 10 BARRACK STREET
(C) CITY:	SYDNEY
(D) STATE:	NEW SOUTH WALES
(E) COUNTRY:	AUSTRALIA
(F) POSTCODE:	2000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:	Floppy disk
(B) COMPUTER:	IBM PC compatible
(C) OPERATING SYSTEM:	PC-DOS/MS-DOS
(D) SOFTWARE:	PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:	Australia
(B) FILING DATE:	

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME:	STEARNE DR, PETER A
(C) REFERENCE	645929/PS

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:	+02 9262 2611
(B) TELEFAX:	+02 9262 1080

(2) INFORMATION FOR SEQ ID No: 1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3336 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 744..3014

(xi) SEQUENCE DESCRIPTION: SEQ ID No 1

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GGTTGGAATC TGGGGTTTTT TTAAATTTAT GCGCTGCTGG CATATAAAAA AAACAACAAC  240
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CCCCACACAT AAAACACTTT TATACTTTTC AAGATCAAAC AAAAGTATAA AAGAAAAAAT  720
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                            1               5

GGC GGT TTT GCC GCT TTA AAA ATG TTA GAA GAA TCC TCC TCA GAA GTA          818
Gly Gly Phe Ala Ala Leu Lys Met Leu Glu Glu Ser Ser Ser Glu Val
 10                  15                  20                  25

ACC TCC TCC TCA AAT GGT CTG GTC TTG TCA TCG GAT ATA AAT ATG TCA          866
Thr Ser Ser Ser Asn Gly Leu Val Leu Ser Ser Asp Ile Asn Met Ser
                 30                  35                  40

CCT TCC TCG TTG GAT TCA CCC GTT TAT GGC GAT CAG GAA ATG TGG CTG          914
Pro Ser Ser Leu Asp Ser Pro Val Tyr Gly Asp Gln Glu Met Trp Leu
             45                  50                  55

TGT AAC GAT TCA GCT TCA TAT AAT AAC AGT CAT CAG CAT AGT GTT ATA          962
Cys Asn Asp Ser Ala Ser Tyr Asn Asn Ser His Gln His Ser Val Ile
         60                  65                  70

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Thr Ser Leu Gln Gly Cys Thr Ser Ser Leu Pro Ala Gln Thr Thr Ile
     75                  80                  85

ATA CCT CTG TCA GCT TTA CCC AAT TCC AAT AAT GCC TCC CTG AAT AAT         1058
Ile Pro Leu Ser Ala Leu Pro Asn Ser Asn Asn Ala Ser Leu Asn Asn
 90                  95                 100                 105

CAA AAT CAA AAT TAT CAA AAT GGT AAT TCC ATG AAT ACA AAT TTA TCG         1106
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GTT AAC ACA AAT AAC AGT GTT GGA GGA GGT GGA GGT GGT GGT GGT GTA         1154
Val Asn Thr Asn Asn Ser Val Gly Gly Gly Gly Gly Gly Gly Gly Val
            125                 130                 135

CCC GGT ATG ACT TCA CTC AAT GGT CTG GGT GGT GGT GGT GGC AGT CAA         1202
Pro Gly Met Thr Ser Leu Asn Gly Leu Gly Gly Gly Gly Gly Ser Gln
        140                 145                 150

GTG AAT AAT CAC AAT CAC AGC CAC AAT CAT TTA CAC CAC AAC AGC AAC         1250
Val Asn Asn His Asn His Ser His Asn His Leu His His Asn Ser Asn
    155                 160                 165

AGT AAT CAC AGT AAT AGC AGT TCC CAC CAC ACA AAT GGC CAC ATG GGT         1298
Ser Asn His Ser Asn Ser Ser Ser His His Thr Asn Gly His Met Gly
170                 175                 180                 185

ATT GGC GGC GGT GGT GGT GGC TTA TCG GTC AAT ATT AAT GGT CCC AAT         1346
Ile Gly Gly Gly Gly Gly Gly Leu Ser Val Asn Ile Asn Gly Pro Asn
                190                 195                 200

ATC GTT AGC AAT GCC CAA CAG TTA AAC TCG TTA CAG GCC TCA CAA AAT         1394
Ile Val Ser Asn Ala Gln Gln Leu Asn Ser Leu Gln Ala Ser Gln Asn
            205                 210                 215
```

```
GGC CAA GTT ATT CAT GCC AAT ATT GGC ATT CAC AGT ATC ATC AGT AAT   1442
Gly Gln Val Ile His Ala Asn Ile Gly Ile His Ser Ile Ile Ser Asn
        220                 225                 230

GGA TTA AAT CAT CAT CAC CAT CAT CAT ATG AAT AAC AGT AGT ATG ATG   1490
Gly Leu Asn His His His His His His Met Asn Asn Ser Ser Met Met
    235                 240                 245

CAT CAT ACA CCC AGA TCT GAA TCA GCT AAT TCC ATA TCA TCA GGT CGT   1538
His His Thr Pro Arg Ser Glu Ser Ala Asn Ser Ile Ser Ser Gly Arg
250                 255                 260                 265

GAT GAT CTT TCA CCC TCG AGC AGT CTT AAT GGC TTC TCA ACA AGC GAT   1586
Asp Asp Leu Ser Pro Ser Ser Ser Leu Asn Gly Phe Ser Thr Ser Asp
                270                 275                 280

GCT AGT GAT GTT AAG AAA ATC AAA AAA GGT CCT GCG CCC CGT TTA CAA   1634
Ala Ser Asp Val Lys Lys Ile Lys Lys Gly Pro Ala Pro Arg Leu Gln
            285                 290                 295

GAG GAA CTG TGT CTG GTG TGT GGT GAT CGG GCG TCC GGT TAT CAT TAT   1682
Glu Glu Leu Cys Leu Val Cys Gly Asp Arg Ala Ser Gly Tyr His Tyr
        300                 305                 310

AAC GCA CTC ACC TGT GAA GGC TGT AAG GGG TTC TTT CGA CGG AGT GTT   1730
Asn Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Val
    315                 320                 325

ACC AAA AAT GCG GTG TAT TGT TGT AAA TTT GGT CAT GCC TGC GAA ATG   1778
Thr Lys Asn Ala Val Tyr Cys Cys Lys Phe Gly His Ala Cys Glu Met
330                 335                 340                 345

GAC ATG TAT ATG CGA CGT AAA TGT CAG GAA TGT AGG CTG AAA AAA TGT   1826
Asp Met Tyr Met Arg Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys
                350                 355                 360

TTG GCT GTG GGC ATG CGG CCG GAA TGT GTG GTG CCC GAA AAC CAG TGT   1874
Leu Ala Val Gly Met Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys
            365                 370                 375

GCA ATG AAA CGA CGC GAA AAG AAA GCA CAA AAA GAG AAG GAT AAA ATA   1922
Ala Met Lys Arg Arg Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Ile
        380                 385                 390

CAG ACC AGT GTG TGT GCA ACG GAA ATT AAA AAG GAA ATA CTC GAT TTA   1970
Gln Thr Ser Val Cys Ala Thr Glu Ile Lys Lys Glu Ile Leu Asp Leu
    395                 400                 405

ATG ACA TGT GAA CCG CCA TCA CAT CCA ACG TGT CCG CTG TTA CCT GAA   2018
Met Thr Cys Glu Pro Pro Ser His Pro Thr Cys Pro Leu Leu Pro Glu
410                 415                 420                 425

GAC ATT TTG GCT AAA TGT CAA GCT CGT AAT ATA CCT CCT TTA TCG TAC   2066
Asp Ile Leu Ala Lys Cys Gln Ala Arg Asn Ile Pro Pro Leu Ser Tyr
                430                 435                 440
```

```
AAT CAA TTG GCA GTT ATA TAT AAA TTA ATA TGG TAT CAA GAT GGC TAC   2114
Asn Gln Leu Ala Val Ile Tyr Lys Leu Ile Trp Tyr Gln Asp Gly Tyr
                445                 450                 455

GAA CAG CCA TCC GAG GAA GAT CTC AAA CGT ATA ATG AGT TCA CCC GAT   2162
Glu Gln Pro Ser Glu Glu Asp Leu Lys Arg Ile Met Ser Ser Pro Asp
        460                 465                 470

GAA AAT GAA AGT CAA CAC GAT GCA TCA TTT CGT CAT ATA ACA GAA ATC   2210
Glu Asn Glu Ser Gln His Asp Ala Ser Phe Arg His Ile Thr Glu Ile
    475                 480                 485

ACT ATA CTA ACA GTA CAA TTA ATT GTG GAA TTT GCC AAG GGT TTG CCA   2258
Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly Leu Pro
490                 495                 500                 505

GCG TTT ACC AAA ATA CCA CAA GAG GAT CAA ATA ACA CTA TTA AAG GCC   2306
Ala Phe Thr Lys Ile Pro Gln Glu Asp Gln Ile Thr Leu Leu Lys Ala
                510                 515                 520

TGC TCA TCA GAA GTT ATG ATG TTG CGA ATG GCA CGA CGT TAC GAT CAC   2354
Cys Ser Ser Glu Val Met Met Leu Arg Met Ala Arg Arg Tyr Asp His
            525                 530                 535

AAT TCA GAT TCG ATA TTC TTT GCC AAT AAT CGA TCG TAT ACG CGT GAC   2402
Asn Ser Asp Ser Ile Phe Phe Ala Asn Asn Arg Ser Tyr Thr Arg Asp
        540                 545                 550

TCT TAT AAA ATG GCT GGC ATG GCT GAT AAT ATT GAG GAT CTG CTG CAT   2450
Ser Tyr Lys Met Ala Gly Met Ala Asp Asn Ile Glu Asp Leu Leu His
    555                 560                 565

TTC TGT CGA CAA ATG TAC TCG ATG AAA GTG GAC AAT GTC GAA TAT GCT   2498
Phe Cys Arg Gln Met Tyr Ser Met Lys Val Asp Asn Val Glu Tyr Ala
570                 575                 580                 585

CTA CTC ACT GCC ATT GTG ATC TTT TCC GAT CGG CCG GGT CTC GAA GAA   2546
Leu Leu Thr Ala Ile Val Ile Phe Ser Asp Arg Pro Gly Leu Glu Glu
                590                 595                 600

GCC GAA CTA GTC GAA GCG ATA CAA AGT TAC TAC ATC GAT ACA CTC CGC   2594
Ala Glu Leu Val Glu Ala Ile Gln Ser Tyr Tyr Ile Asp Thr Leu Arg
            605                 610                 615

ATT TAC ATA CTT AAT CGC CAT TGC GGC GAT CCC ATG AGT CTC GTA TTC   2642
Ile Tyr Ile Leu Asn Arg His Cys Gly Asp Pro Met Ser Leu Val Phe
        620                 625                 630

TTT GCC AAG CTT CTG TCA ATT CTA ACC GAA CTG CGT ACG TTG GGC AAT   2690
Phe Ala Lys Leu Leu Ser Ile Leu Thr Glu Leu Arg Thr Leu Gly Asn
    635                 640                 645

CAA AAT GCC GAA ATG TGT TTC TCG TTG AAA TTG AAA AAT CGC AAA CTG   2738
Gln Asn Ala Glu Met Cys Phe Ser Leu Lys Leu Lys Asn Arg Lys Leu
650                 655                 660                 665
```

```
CCA AAA TTC CTC GAA GAG ATC TGG GAT GTA CAT GCC ATT CCA CCC TCA   2786
Pro Lys Phe Leu Glu Glu Ile Trp Asp Val His Ala Ile Pro Pro Ser
                670                 675                 680

GTG CAG TCA CAC ATA CAG GCT ACC CAG GCG GAA AAG GCC GGC CCA GGA   2834
Val Gln Ser His Ile Gln Ala Thr Gln Ala Glu Lys Ala Gly Pro Gly
            685                 690                 695

AGC TCA GGC AAC AAC ATC GGC CAT TTC AGC AGC CGC CAC CTC ATC TTC   2882
Ser Ser Gly Asn Asn Ile Gly His Phe Ser Ser Arg His Leu Ile Phe
        700                 705                 710

CTC CAT AAA TAC CTC GAT GGC AAC ATC ATC CTC ATC ATC GTT ATC GCC   2930
Leu His Lys Tyr Leu Asp Gly Asn Ile Ile Leu Ile Ile Val Ile Ala
    715                 720                 725

ATC GGC GCC TCA ACA CCC AAT GGT GGT GCC GTC GAT TAT GTT GGC ACC   2978
Ile Gly Ala Ser Thr Pro Asn Gly Gly Ala Val Asp Tyr Val Gly Thr
730                 735                 740                 745

GAT ATG AGT ATG AGT TTA GTA CAA TCG GAT AAT GCA TAGCAATAGC        3024
Asp Met Ser Met Ser Leu Val Gln Ser Asp Asn Ala
                750                 755

TTTTAACAAC TACTACTATT GCCAACGAAG AGAAGAGTGC TGATTGTGGT GGTAGTGTTA 3084

ATATCGTCCC TGAGATAGTA GCTGACATTG AAGAGACGTT GATGATAATG ATGTTGTTGA 3144

TGACGGTGAT GATGACGATG TTGTTGATGA TGATGTGACA ATGAGAGAGT TGTGTTATTA 3204

AATACTTCTT CTATTTCAAG TGGCTGTTAA CTTTATCCAA CATCATCATA AGTTGGAATA 3264

GAAAAGTGAT GAAAATTAAT AGATCAAGAG ACAGAAACCG CAAGTGACAA ATTAAACAAA 3324

AAAAAAAAAA AA                                                     3336
```

(2) INFORMATION FOR SEQ ID No: 2

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 757 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID No 2

```
Met Met Lys Arg Arg Trp Ser Asn Asn Gly Gly Phe Ala Ala Leu Lys
  1               5                  10                  15

Met Leu Glu Glu Ser Ser Ser Glu Val Thr Ser Ser Ser Asn Gly Leu
            20                  25                  30
```

Val Leu Ser Ser Asp Ile Asn Met Ser Pro Ser Ser Leu Asp Ser Pro
35 40 45

Val Tyr Gly Asp Gln Glu Met Trp Leu Cys Asn Asp Ser Ala Ser Tyr
50 55 60

Asn Asn Ser His Gln His Ser Val Ile Thr Ser Leu Gln Gly Cys Thr
65 70 75 80

Ser Ser Leu Pro Ala Gln Thr Thr Ile Ile Pro Leu Ser Ala Leu Pro
85 90 95

Asn Ser Asn Asn Ala Ser Leu Asn Asn Gln Asn Gln Asn Tyr Gln Asn
100 105 110

Gly Asn Ser Met Asn Thr Asn Leu Ser Val Asn Thr Asn Asn Ser Val
115 120 125

Gly Gly Gly Gly Gly Gly Gly Gly Val Pro Gly Met Thr Ser Leu Asn
130 135 140

Gly Leu Gly Gly Gly Gly Gly Ser Gln Val Asn Asn His Asn His Ser
145 150 155 160

His Asn His Leu His His Asn Ser Asn Ser Asn His Ser Asn Ser Ser
165 170 175

Ser His His Thr Asn Gly His Met Gly Ile Gly Gly Gly Gly Gly Gly
180 185 190

Leu Ser Val Asn Ile Asn Gly Pro Asn Ile Val Ser Asn Ala Gln Gln
195 200 205

Leu Asn Ser Leu Gln Ala Ser Gln Asn Gly Gln Val Ile His Ala Asn
210 215 220

Ile Gly Ile His Ser Ile Ile Ser Asn Gly Leu Asn His His His His
225 230 235 240

His His Met Asn Asn Ser Ser Met Met His His Thr Pro Arg Ser Glu
245 250 255

Ser Ala Asn Ser Ile Ser Ser Gly Arg Asp Asp Leu Ser Pro Ser Ser
260 265 270

Ser Leu Asn Gly Phe Ser Thr Ser Asp Ala Ser Asp Val Lys Lys Ile
275 280 285

Lys Lys Gly Pro Ala Pro Arg Leu Gln Glu Glu Leu Cys Leu Val Cys
290 295 300

Gly Asp Arg Ala Ser Gly Tyr His Tyr Asn Ala Leu Thr Cys Glu Gly
305 310 315 320

Cys Lys Gly Phe Phe Arg Arg Ser Val Thr Lys Asn Ala Val Tyr Cys
325 330 335

```
Cys Lys Phe Gly His Ala Cys Glu Met Asp Met Tyr Met Arg Arg Lys
            340                 345                 350

Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Ala Val Gly Met Arg Pro
        355                 360                 365

Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg Arg Glu Lys
    370                 375                 380

Lys Ala Gln Lys Glu Lys Asp Lys Ile Gln Thr Ser Val Cys Ala Thr
385                 390                 395                 400

Glu Ile Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Ser
                405                 410                 415

His Pro Thr Cys Pro Leu Leu Pro Glu Asp Ile Leu Ala Lys Cys Gln
            420                 425                 430

Ala Arg Asn Ile Pro Pro Leu Ser Tyr Asn Gln Leu Ala Val Ile Tyr
        435                 440                 445

Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Glu Glu Asp
    450                 455                 460

Leu Lys Arg Ile Met Ser Ser Pro Asp Glu Asn Glu Ser Gln His Asp
465                 470                 475                 480

Ala Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr Val Gln Leu
                485                 490                 495

Ile Val Glu Phe Ala Lys Gly Leu Pro Ala Phe Thr Lys Ile Pro Gln
            500                 505                 510

Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met
        515                 520                 525

Leu Arg Met Ala Arg Arg Tyr Asp His Asn Ser Asp Ser Ile Phe Phe
    530                 535                 540

Ala Asn Asn Arg Ser Tyr Thr Arg Asp Ser Tyr Lys Met Ala Gly Met
545                 550                 555                 560

Ala Asp Asn Ile Glu Asp Leu Leu His Phe Cys Arg Gln Met Tyr Ser
                565                 570                 575

Met Lys Val Asp Asn Val Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile
            580                 585                 590

Phe Ser Asp Arg Pro Gly Leu Glu Glu Ala Glu Leu Val Glu Ala Ile
        595                 600                 605

Gln Ser Tyr Tyr Ile Asp Thr Leu Arg Ile Tyr Ile Leu Asn Arg His
    610                 615                 620

Cys Gly Asp Pro Met Ser Leu Val Phe Phe Ala Lys Leu Leu Ser Ile
625                 630                 635                 640
```

```
Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ala Glu Met Cys Phe
                645                 650                 655

Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Lys Phe Leu Glu Glu Ile
            660                 665                 670

Trp Asp Val His Ala Ile Pro Pro Ser Val Gln Ser His Ile Gln Ala
        675                 680                 685

Thr Gln Ala Glu Lys Ala Gly Pro Gly Ser Ser Gly Asn Asn Ile Gly
    690                 695                 700

His Phe Ser Ser Arg His Leu Ile Phe Leu His Lys Tyr Leu Asp Gly
705                 710                 715                 720

Asn Ile Ile Leu Ile Ile Val Ile Ala Ile Gly Ala Ser Thr Pro Asn
                725                 730                 735

Gly Gly Ala Val Asp Tyr Val Gly Thr Asp Met Ser Met Ser Leu Val
            740                 745                 750

Gln Ser Asp Asn Ala
        755
```

(2) INFORMATION FOR SEQ ID No: 3

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1398 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1398

(xi) SEQUENCE DESCRIPTION: SEQ ID No 3

```
ATG GAT AAC GGC GAG CAA GAT GCT GGG TTC CGA TTG GCA CCG ATG TCT      48
Met Asp Asn Gly Glu Gln Asp Ala Gly Phe Arg Leu Ala Pro Met Ser
  1               5                  10                  15

CCG CAG GAG ATA AAG CCA GAC ATT TCA CTA CTC AAT GAA AAT AAT ACG      96
Pro Gln Glu Ile Lys Pro Asp Ile Ser Leu Leu Asn Glu Asn Asn Thr
            20                  25                  30

AGT AGT TAT TCG CCC AAA CCT GGA AGT CCT AAT CCA TTT GCC ATC GGA     144
Ser Ser Tyr Ser Pro Lys Pro Gly Ser Pro Asn Pro Phe Ala Ile Gly
        35                  40                  45
```

```
TTG CAG GCA ATA AAT GCA GTC GCT GCC GCG AAT GCC AAT AAC CAA AAT     192
Leu Gln Ala Ile Asn Ala Val Ala Ala Ala Asn Ala Asn Asn Gln Asn
     50                  55                  60

CAA ATG TTG CAA ACT ACG CCA CCA CAA CAG CAG CAG TAT CCA CCA AAT     240
Gln Met Leu Gln Thr Thr Pro Pro Gln Gln Gln Gln Tyr Pro Pro Asn
 65                  70                  75                  80

CAC CCC CTT AGT GGT TCG AAA CAC TTG TGT TCC ATT TGT GGA GAC CGC     288
His Pro Leu Ser Gly Ser Lys His Leu Cys Ser Ile Cys Gly Asp Arg
                 85                  90                  95

GCC AGT GGA AAA CAT TAT GGG GTC TAC AGT TGT GAG GGT TGT AAA GGG     336
Ala Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly
            100                 105                 110

TTC TTC AAA CGT ACC GTA CGC AAG GAC TTG ACA TAT GCT TGT CGT GAG     384
Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Ala Cys Arg Glu
        115                 120                 125

GAC AGA AAT TGC ATT ATT GAT AAA CGA CAA AGA AAT CGT TGC CAG TAT     432
Asp Arg Asn Cys Ile Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr
    130                 135                 140

TGT CGT TAT CAA AAG TGT TTA GCT TGT GGC ATG AAA CGC GAA GCG GTC     480
Cys Arg Tyr Gln Lys Cys Leu Ala Cys Gly Met Lys Arg Glu Ala Val
145                 150                 155                 160

CAA GAG GAA CGA CAA CGT GGT ACT CGT GCT GCT AAC GCT AGA GCT GCT     528
Gln Glu Glu Arg Gln Arg Gly Thr Arg Ala Ala Asn Ala Arg Ala Ala
                165                 170                 175

GGT GCT GGC GGT GGT GGA GGA GGT GGT GGT GGG GTA AGC AAT GTG GTT     576
Gly Ala Gly Gly Gly Gly Gly Gly Gly Gly Gly Val Ser Asn Val Val
            180                 185                 190

GGT GCT GGC GGA GAA GAC TTT AAA CCC AGC AGT TCA TTA CGT GAT CTC     624
Gly Ala Gly Gly Glu Asp Phe Lys Pro Ser Ser Ser Leu Arg Asp Leu
        195                 200                 205

ACT ATA GAA CGC ATC ATT GAA GCC GAG CAA AAG GCT GAA TCT TTG AGC     672
Thr Ile Glu Arg Ile Ile Glu Ala Glu Gln Lys Ala Glu Ser Leu Ser
    210                 215                 220

GGT GAT AAC GTG TTG CCC TTT TTG CGC GTT GGC AAC AAT TCC ATG GTA     720
Gly Asp Asn Val Leu Pro Phe Leu Arg Val Gly Asn Asn Ser Met Val
225                 230                 235                 240

CAA CAC GAC TAC AAA GGC GCG GTA TCT CAT CTC TGC CAG ATG GTT AAC     768
Gln His Asp Tyr Lys Gly Ala Val Ser His Leu Cys Gln Met Val Asn
                245                 250                 255

AAA CAA CTC TAC CAA ATG GTT GAA TAT GCA CGT CGA ACA CCA CAT TTT     816
Lys Gln Leu Tyr Gln Met Val Glu Tyr Ala Arg Arg Thr Pro His Phe
            260                 265                 270
```

```
ACA CAT TTG CAG CGT GAG GAT CAG ATA CTA TTG TTA AAG GCT GGC TGG     864
Thr His Leu Gln Arg Glu Asp Gln Ile Leu Leu Leu Lys Ala Gly Trp
        275                 280                 285

AAT GAA CTG CTA ATT GCA AAT GTT GCC TGG TGC AGT ATT GAG TCT CTG     912
Asn Glu Leu Leu Ile Ala Asn Val Ala Trp Cys Ser Ile Glu Ser Leu
    290                 295                 300

GAT GCC GAA TAT GCC TCT CCT GGT ACG GTA CAT GAC GGT TCT TTT GGT     960
Asp Ala Glu Tyr Ala Ser Pro Gly Thr Val His Asp Gly Ser Phe Gly
305                 310                 315                 320

CGG CGT TCA CCA GTG CGT CAG CCC CAA CAA CTC TTC CTT AAT CAG AAT    1008
Arg Arg Ser Pro Val Arg Gln Pro Gln Gln Leu Phe Leu Asn Gln Asn
                325                 330                 335

TTC TCG TAT CAT CGC AAT AGT GCT ATT AAG GCC AAT GTT GTT TCA ATT    1056
Phe Ser Tyr His Arg Asn Ser Ala Ile Lys Ala Asn Val Val Ser Ile
            340                 345                 350

TTC GAT CGT ATC CTC TCG GAG TTG AGC ATC AAA ATG AAA CGT CTT AAC    1104
Phe Asp Arg Ile Leu Ser Glu Leu Ser Ile Lys Met Lys Arg Leu Asn
        355                 360                 365

ATC GAT CGC TCG GAG TTG TCG TGT CTG AAG GCA ATC ATA CTC TTC AAT    1152
Ile Asp Arg Ser Glu Leu Ser Cys Leu Lys Ala Ile Ile Leu Phe Asn
    370                 375                 380

CCA GAC ATA CGC GGT CTG AAA TGT CGA GCC GAC GTC GAG GTA TGT CGT    1200
Pro Asp Ile Arg Gly Leu Lys Cys Arg Ala Asp Val Glu Val Cys Arg
385                 390                 395                 400

GAA AAA ATC TAT GCC TGT CTG GAC GAA CAC TGC CGC ACA GAA CAT CCA    1248
Glu Lys Ile Tyr Ala Cys Leu Asp Glu His Cys Arg Thr Glu His Pro
                405                 410                 415

GGT GAT GAT GGC CGC TTT GCT CAG CTA CTA CTA AGG TTG CCC GCA TTG    1296
Gly Asp Asp Gly Arg Phe Ala Gln Leu Leu Leu Arg Leu Pro Ala Leu
            420                 425                 430

CTT CCA TCA ATC TCA AAT GTC TCG ATC ATT TGT TTT CCT CCG TTT AAT    1344
Leu Pro Ser Ile Ser Asn Val Ser Ile Ile Cys Phe Pro Pro Phe Asn
        435                 440                 445

AGG CGA AAA ACA TTG GAG GAA TTA ATG CTG AAC AAT TGG AAC CCC ATC    1392
Arg Arg Lys Thr Leu Glu Glu Leu Met Leu Asn Asn Trp Asn Pro Ile
    450                 455                 460

TGC TAA                                                            1398
Cys
465
```

(2) INFORMATION FOR SEQ ID No: 4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 465 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID No 4

```
Met Asp Asn Gly Glu Gln Asp Ala Gly Phe Arg Leu Ala Pro Met Ser
 1               5                  10                  15

Pro Gln Glu Ile Lys Pro Asp Ile Ser Leu Leu Asn Glu Asn Asn Thr
            20                  25                  30

Ser Ser Tyr Ser Pro Lys Pro Gly Ser Pro Asn Pro Phe Ala Ile Gly
        35                  40                  45

Leu Gln Ala Ile Asn Ala Val Ala Ala Ala Asn Ala Asn Asn Gln Asn
    50                  55                  60

Gln Met Leu Gln Thr Thr Pro Pro Gln Gln Gln Gln Tyr Pro Pro Asn
65                  70                  75                  80

His Pro Leu Ser Gly Ser Lys His Leu Cys Ser Ile Cys Gly Asp Arg
                85                  90                  95

Ala Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly
            100                 105                 110

Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Ala Cys Arg Glu
        115                 120                 125

Asp Arg Asn Cys Ile Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr
    130                 135                 140

Cys Arg Tyr Gln Lys Cys Leu Ala Cys Gly Met Lys Arg Glu Ala Val
145                 150                 155                 160

Gln Glu Glu Arg Gln Arg Gly Thr Arg Ala Ala Asn Ala Arg Ala Ala
                165                 170                 175

Gly Ala Gly Gly Gly Gly Gly Gly Gly Gly Gly Val Ser Asn Val Val
            180                 185                 190

Gly Ala Gly Gly Glu Asp Phe Lys Pro Ser Ser Ser Leu Arg Asp Leu
        195                 200                 205

Thr Ile Glu Arg Ile Ile Glu Ala Glu Gln Lys Ala Glu Ser Leu Ser
    210                 215                 220

Gly Asp Asn Val Leu Pro Phe Leu Arg Val Gly Asn Asn Ser Met Val
225                 230                 235                 240
```

```
Gln His Asp Tyr Lys Gly Ala Val Ser His Leu Cys Gln Met Val Asn
                245                 250                 255

Lys Gln Leu Tyr Gln Met Val Glu Tyr Ala Arg Arg Thr Pro His Phe
            260                 265                 270

Thr His Leu Gln Arg Glu Asp Gln Ile Leu Leu Leu Lys Ala Gly Trp
        275                 280                 285

Asn Glu Leu Leu Ile Ala Asn Val Ala Trp Cys Ser Ile Glu Ser Leu
    290                 295                 300

Asp Ala Glu Tyr Ala Ser Pro Gly Thr Val His Asp Gly Ser Phe Gly
305                 310                 315                 320

Arg Arg Ser Pro Val Arg Gln Pro Gln Gln Leu Phe Leu Asn Gln Asn
                325                 330                 335

Phe Ser Tyr His Arg Asn Ser Ala Ile Lys Ala Asn Val Val Ser Ile
            340                 345                 350

Phe Asp Arg Ile Leu Ser Glu Leu Ser Ile Lys Met Lys Arg Leu Asn
        355                 360                 365

Ile Asp Arg Ser Glu Leu Ser Cys Leu Lys Ala Ile Ile Leu Phe Asn
    370                 375                 380

Pro Asp Ile Arg Gly Leu Lys Cys Arg Ala Asp Val Glu Val Cys Arg
385                 390                 395                 400

Glu Lys Ile Tyr Ala Cys Leu Asp Glu His Cys Arg Thr Glu His Pro
                405                 410                 415

Gly Asp Asp Gly Arg Phe Ala Gln Leu Leu Leu Arg Leu Pro Ala Leu
            420                 425                 430

Leu Pro Ser Ile Ser Asn Val Ser Ile Ile Cys Phe Pro Pro Phe Asn
        435                 440                 445

Arg Arg Lys Thr Leu Glu Glu Leu Met Leu Asn Asn Trp Asn Pro Ile
    450                 455                 460

Cys
465
```

(2) INFORMATION FOR SEQ ID No: 5

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 561 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..561

(xi) SEQUENCE DESCRIPTION: SEQ ID No 5

```
TGT GAA GGC TGT AAG GGT TTC TTT CGA CGG AGT GTT ACC AAA AAT GCG      48
Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Val Thr Lys Asn Ala
  1               5                  10                  15

GTG TAT TGT TGT AAA TTT GGT CAT GCC TGC GAA ATG GAC ATG TAT ATG      96
Val Tyr Cys Cys Lys Phe Gly His Ala Cys Glu Met Asp Met Tyr Met
            20                  25                  30

CGA CGT AAA TGT CAG GAA TGT AGG CTG AAA AAA TGT TTG GCT GTG GGC     144
Arg Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Ala Val Gly
        35                  40                  45

ATG CGG CCG GAA TGT GTG GTG CCC GAA AAC CAG TGT GCA ATG AAA CGA     192
Met Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg
    50                  55                  60

CGC GAA AAG AAA GCA CAA AAA GAG AAG GAT AAA ATA CAG ACC AGT GTG     240
Arg Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Ile Gln Thr Ser Val
65                  70                  75                  80

TGT GCA ACG GAA ATT AAA AAG GAA ATA CTC GAT TTA ATG ACA TGT GAA     288
Cys Ala Thr Glu Ile Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu
                85                  90                  95

CCG CCA TCA CAT CCA ACG TGT CCG CTG TTA CCT GAA GAC ATT TTG GCT     336
Pro Pro Ser His Pro Thr Cys Pro Leu Leu Pro Glu Asp Ile Leu Ala
            100                 105                 110

AAA TGT CAA GCT CGT AAT ATA CCT CCT TTA TCG TAC AAT CAA TTG GCA     384
Lys Cys Gln Ala Arg Asn Ile Pro Pro Leu Ser Tyr Asn Gln Leu Ala
        115                 120                 125

GTT ATA TAT AAA TTA ATA TGG TAT CAA GAT GGC TAC GAA CAG CCA TCC     432
Val Ile Tyr Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser
    130                 135                 140
```

```
GAG GAA GAT CTC AAA CGT ATA ATG AGT TCA CCC GAT GAA AAT GAA AGT     480
Glu Glu Asp Leu Lys Arg Ile Met Ser Ser Pro Asp Glu Asn Glu Ser
145                 150                 155                 160

CAA CAC GAT GCA TCA TTT CGT CAT ATA ACA GAA ATC ACT ATA CTA ACA     528
Gln His Asp Ala Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr
                165                 170                 175

GTA CAA TTA ATT GTT GAA TGT GCC AAA GGT CTA                        561
Val Gln Leu Ile Val Glu Cys Ala Lys Gly Leu
            180                 185
```

(2) INFORMATION FOR SEQ ID No: 6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 187 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID No 6

```
Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Val Thr Lys Asn Ala
 1               5                  10                  15

Val Tyr Cys Cys Lys Phe Gly His Ala Cys Glu Met Asp Met Tyr Met
            20                  25                  30

Arg Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Ala Val Gly
        35                  40                  45

Met Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg
    50                  55                  60

Arg Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Ile Gln Thr Ser Val
65                  70                  75                  80

Cys Ala Thr Glu Ile Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu
                85                  90                  95

Pro Pro Ser His Pro Thr Cys Pro Leu Leu Pro Glu Asp Ile Leu Ala
            100                 105                 110

Lys Cys Gln Ala Arg Asn Ile Pro Pro Leu Ser Tyr Asn Gln Leu Ala
        115                 120                 125

Val Ile Tyr Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser
    130                 135                 140

Glu Glu Asp Leu Lys Arg Ile Met Ser Ser Pro Asp Glu Asn Glu Ser
145                 150                 155                 160
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Gln His Asp Ala Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr
165 170 175

Val Gln Leu Ile Val Glu Cys Ala Lys Gly Leu
180 185

The claims defining the invention are as follows:

1. A screening system for insecticidally active agents comprising a nucleotide sequence encoding an insect steroid receptor or a fragment thereof, and a nucleotide sequence encoding a partner protein or a fragment thereof which associates with the receptor so as to confer enhanced affinity for insect steroid response elements, enhanced affinity for insect steroids or analogues thereof, or insecticidally active agents and/or thermostability or enhanced thermostability of said receptor, which receptor and partner protein is capable of binding to a candidate insecticidally active agent to form an activated complex, and a nucleic acid sequence encoding a bioactive molecule or a reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex regulates transcription of the nucleic acid sequence, wherein on exposure to said agent expression of the bioactive molecule or reporter molecule correlates with insecticidal activity.

2. A screening system according to claim 1 which comprises a prokaryotic or eukaryotic cell, a cell lysate, or an aqueous solution.

3. A screening system according to claim 1 wherein said bioactive molecule or reporter is a peptide or protein.

4. A screening system according to claim 3 which comprises a prokaryotic or eukaryotic cell.

5. A screening system according to claim 1 wherein said thermostable insect steroid receptor is an ecdysteroid receptor from organisms of the classes insecta, cestoda, trematoda, nematoda, and protozoa.

6. A screening system according to claim 5 wherein said organisms selected from the Australian sheep blowfly, hemiptera (such as aphid, scale insect, and leaf hopper), beetle, moth, ant, helminth and protozoan.

7. A screening system according to claim 1 wherein said nucleotide sequence encoding a thermostable insect steroid receptor encodes the *Lucilia* ecdysteroid receptor having the nucleotide sequence identified as SEQ ID: 1.

8. A screening system according to claim 1 wherein said one or more insect steroid response elements are located within a promoter.

9. A screening system according to claim 8 wherein a plurality of insect steroid response elements are located within the promoter.

10. A screening system according to claim 8 wherein said insect steroid response elements replace sequences within a selected promoter which confer responsiveness to hormones which regulate promoter activity.

11. A screening system according to claim 9 wherein said response elements may be same or different and when different are selected so as to bind different insect steroids or analogues thereof such that the promoter may be differentially regulated.

12. A method for the regulated production of a bioactive molecule or a reporter molecule in a cell, said method comprising the steps of introducing into said cell:

a) a nucleotide sequence encoding an insect steroid receptor or a fragment thereof which is capable of binding an insect steroid or analogue thereof, to form an activated complex; and

b) a nucleic acid sequence encoding said bioactive molecule or reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex regulates transcription of the nucleic acid sequence encoding said bioactive molecule or reporter molecule,

wherein exposing the cell to an insect steroid or analogue thereof regulates expression of the bioactive molecule or reporter molecule.

13. A method according to claim 12 wherein said bioactive molecule or reporter molecule is a peptide or polypeptide.

14. A method according to claim 12 wherein said thermostable insect steroid receptor is an ecdysteroid receptor from organisms of the class insecta, cestoda, trematoda, menatoda, and protozoa.

15. A method according to claim 14 wherein said organisms of the class insecta are selected from the Australian sheep blowfly, hemiptera (such as aphid, scale insect, and leaf hopper), beetle, moth, ant, helminth and protozoan.

16. A method according to claim 12 wherein said nucleotide sequence encoding an insect steroid receptor encodes the *Lucilia* ecdysteroid receptor having the nucleotide sequence identified as SEQ ID: 1.

17. A method according to claim 12 wherein said one or more insect steroid response elements are located within a promoter.

18. A method according to claim 17 wherein a plurality of insect steroid response elements are located within the promoter.

19. A method according to claim 17 wherein said insect steroid response elements replace sequences within a promoter which lead to responsiveness to hormones which regulate promoter activity.

20. A method according to claim 18 wherein said response elements may be same or different and when different are selected so as to bind different insect steroids or analogues thereof such that the promoter may be differentially regulated.

21. A method according to claim 12 which additionally comprises introducing into said cell a nucleotide sequence encoding a partner protein or a fragment thereof which associates with the receptor so as to confer enhanced affinity for insect steroid response elements, enhanced affinity for insect steroids or analogues thereof or insecticidally active agents, and/or thermostability or enhanced thermostability of said receptor.

22. A method according to claim 12 wherein said cell is a human liver cell.

23. A method according to claim 12 wherein said bioactive molecule is insulin.

24. A cell which expresses an insect steroid receptor polypeptide or a fragment thereof which receptor is capable of binding to an insect steroid or analogue thereof or a candidate insecticidally active agent to form an activated complex, and a nucleic acid sequence encoding a bioactive molecule or a reporter molecule operably linked to one or more insect steroid response elements which on binding of the said activated complex promotes transcription of the nucleic acid sequence, wherein said cell on exposure to insect steroid or an analogue thereof, regulates expression of said bioactive molecule or allows detection of said reporter molecule.

25. A cell according to claim 24 wherein said insect steroid receptor is capable of binding an ecdysteroid or an analogue thereof.

26. A cell according to claim 24 wherein said receptor is an insect steroid receptor selected from the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, moth, ant, helminth or protozoan.

27. A cell according to claim 24 wherein said one or more insect steroid response elements are located within a promoter.

28. A cell according to claim 27 wherein a plurality of insect steroid response elements are located within the promoter.

29. A cell according to claim 27 wherein said insect steroid response elements replace sequences within a promoter which lead to responsiveness to hormones which regulate promoter activity.

30. A cell according to claim 28 wherein said response elements may be same or different and when different are selected so as to lead to differential binding of different insect steroids or analogues thereof such that the promoter may be differentially regulated.

31. A cell according to claim 26 which additionally expresses a partner protein which associates with the receptor so as to confer enhanced affinity for insect steroid response elements; enhanced affinity for insect steroids or analogues thereof, or insecticidally active agents and/or enhanced thermostability of said receptor.

32. A cell according to claim 24 which is a prokaryotic or eukaryotic cell.

33. An isolated recombinant nucleic acid sequence encoding an insect steroid receptor selected from the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, moth, ant, helminth or protozoan or a fragment thereof capable of binding an insect steroid, an analogue thereof, or an insecticidally active agent.

34. A nucleic acid sequence according to claim 33 to which comprises SEQ ID NO: 1 or SEQ ID NO: 5.

35 A recombinant nucleic acid comprising one or more insect steroid response elements from the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, ant, helminth or protozoan capable of binding to an insect steroid receptor and operably linked to a promoter sequence or located within a promoter sequence which in turn is operably linked to a DNA sequence encoding a bioactive molecule.

36. A recombinant nucleic acid according to claim 35 wherein said one or more insect steroid response elements are located within a promoter.

37. A recombinant nucleic acid according to claim 35 wherein a plurality of insect steroid response elements are located within the promoter.

38. A recombinant nucleic acid according to claim 35 wherein said insect steroid response elements replace sequences within a promoter which are responsive to hormones which regulate promoter activity.

39. A recombinant nucleic acid according to claim 37 wherein said response elements may be same or different and when different are selected so as to lead to differential binding different insect steroids or analogues thereof such that the promoter may be differentially regulated.

40. A polypeptide comprising an insect steroid receptor or fragment thereof from the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, moth, ant, helminth or protozoan, which polypeptide is substantially free of naturally associated insect cell components.

41. A partner polyopeptide or a fragment thereof from the Australian sheep blowfly, hemiptera (such as aphid, scale insect and leaf hopper), beetle, moth, ant, helminth or protozoan which associates with an insect receptor so as to confer enhanced affinity for insect steroid response elements, enhanced affinity for insect steroids or analogous thereof or insecticidally active agents, and/or thermostability or enhanced thermostability of said receptor.

42. A polypeptide according to claim 41 which comprises the amino acid sequence set forth in SEQ ID: 2.

43. A screening system according to any one of claims 1 to 11, a method according to any one of claims 12 to 23, a cell according to any one of claims 24 to 32, a nucleic acid sequence according to any one of claims 33 to 39, or a polypeptide according to any one of claims 40 to 42, wherein said insect steroid receptor is thermostable.

44. Factors which associate with insect steroid receptors and which confer enhanced affinity for insect steroids or analogues thereof or insecticidally active agents, enhanced affinity for insect steroid response elements, and/or thermostability or enhanced thermostability of said receptors.

45. A method or assay for screening insecticidally active compounds utilising an insect steroid receptor polypeptide or a fragment thereof encompassing the ligand binding domain, or a complex thereof with a partner protein or a fragment thereof encompassing the ligand binding domain which confers enhanced affinity for insect steroid response elements, enhanced affinity for insect steroids or compounds which bind said receptor which comprises reacting the protein or complex thereof with a candidate insecticidally active molecule, and thereafter detecting binding or absence of binding of said compound so as to determine insecticidal activity.

46. A synthetic compound derived from the three dimensional structure of an insect steroid receptor as hereinbefore described which compounds are capable of binding to said receptors and which have the effect of either inactivating the receptors or potentiating the activity thereof.

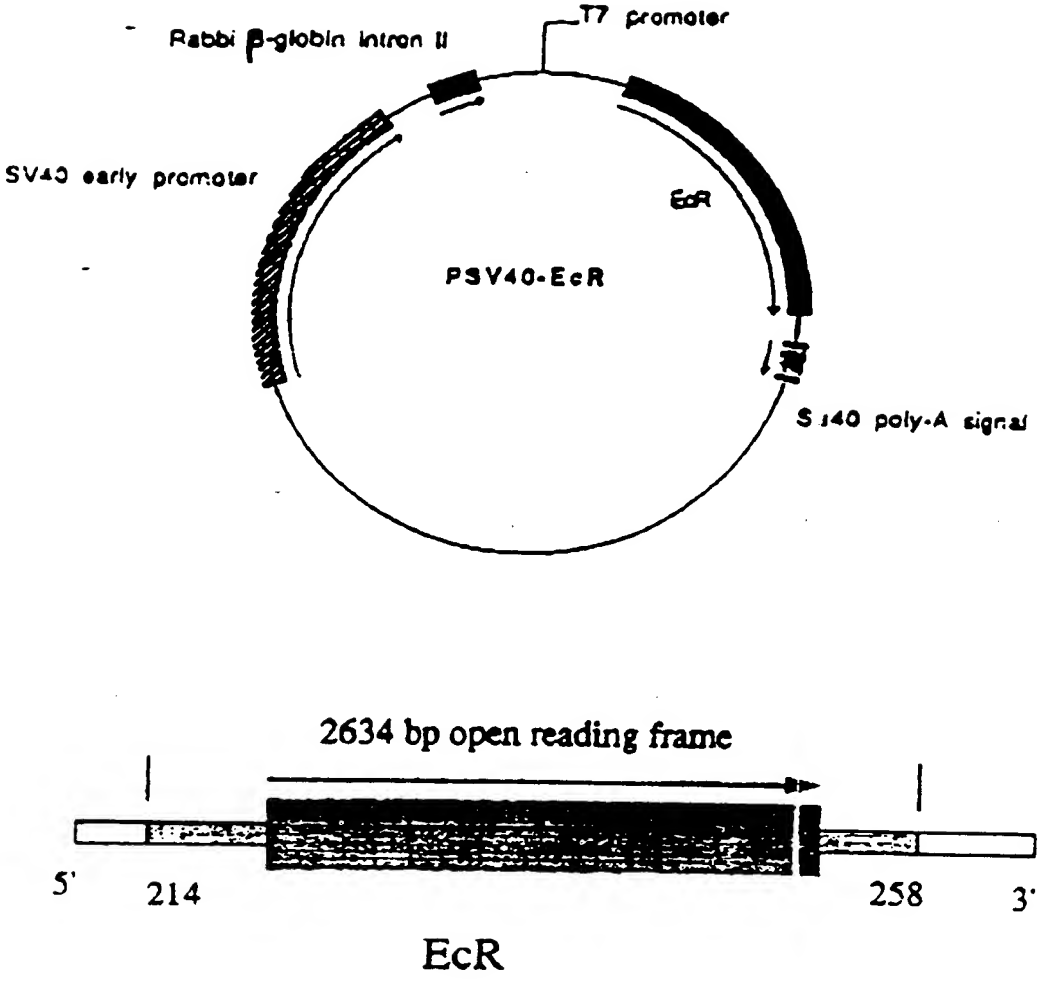
47. A method for the determination/production of insecticidally active agents which comprises the steps of:

a) expression and purification of an insect steroid receptor or a fragment encompassing the ligand binding domain thereof optionally in association with a partner protein or ligand binding domain thereof, optionally in association with an insect steroid or analogue thereof so as to form a complex;

b) determining the three dimensional crystal structure of said complex and determining thereafter the three dimensional structure of the ligand binding domain; and

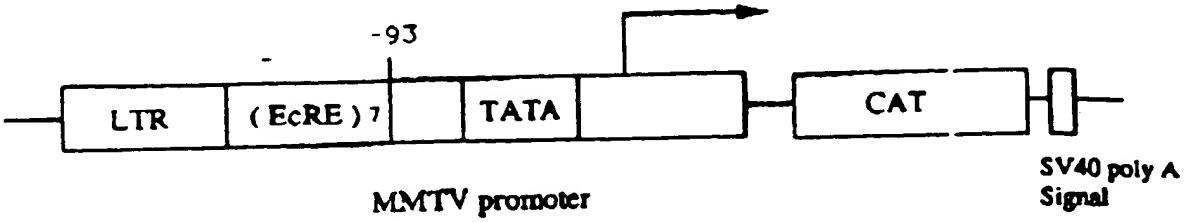
c) synthesising compounds which bind to or associate with the ligand binding domain.

FIGURE 1:



Structure of Ecdysone Receptor Expression Plasmid pSV40-EcR

FIGURE 2:



Structure of Reporter Plasmid p(EcRE)7-CAT

FIGURE 3:

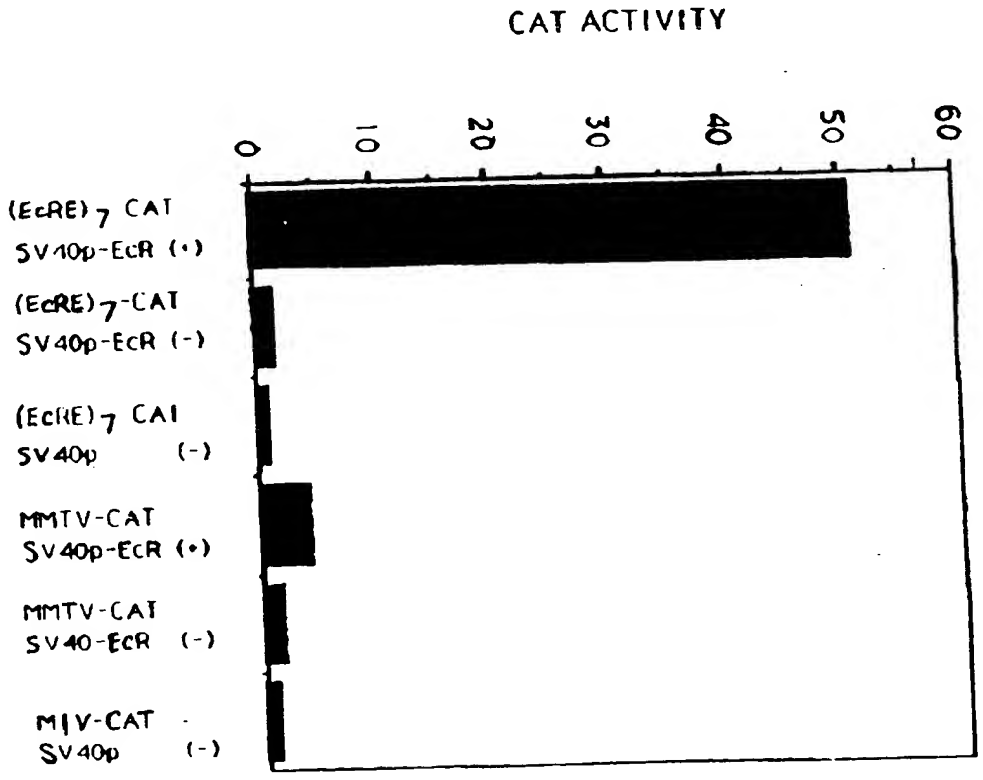
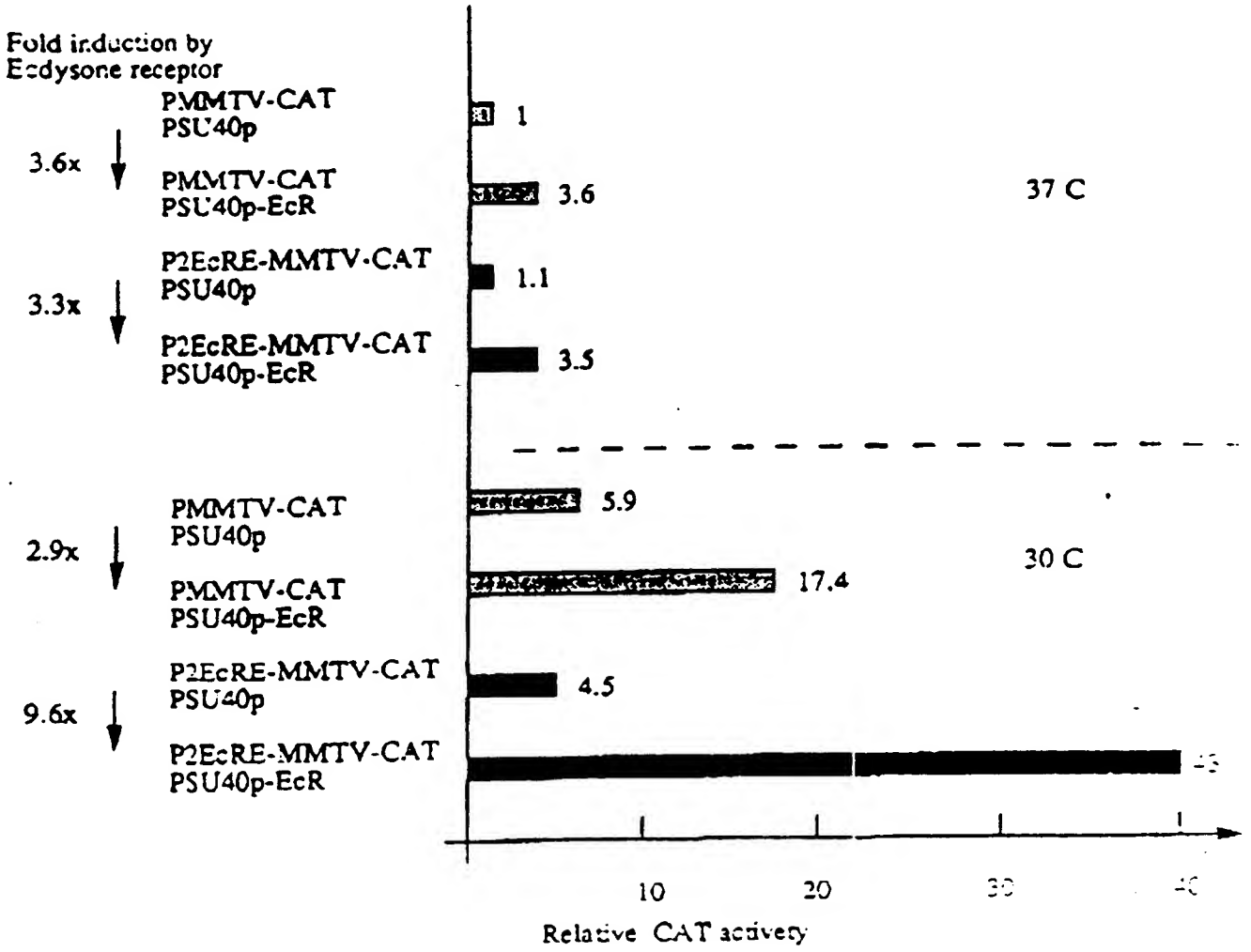


FIGURE 4:

TEMPERATURE EFECT ON REPORTER GENE INDUCTION BY PNA

pSV40-EcR µgm/dish	PNA µM	Temperature 37°	30°
2.5	20	14X	35X
	100	59X	54X
0.5	20	8X	26X
	100	47X	33X
0.1	20	1.6X	25X
	100	9.0X	39X

FIGURE 5:



Comperision of ecdysone receptor function at 30 C and 37 C in CHO cell

FIGURE 6:

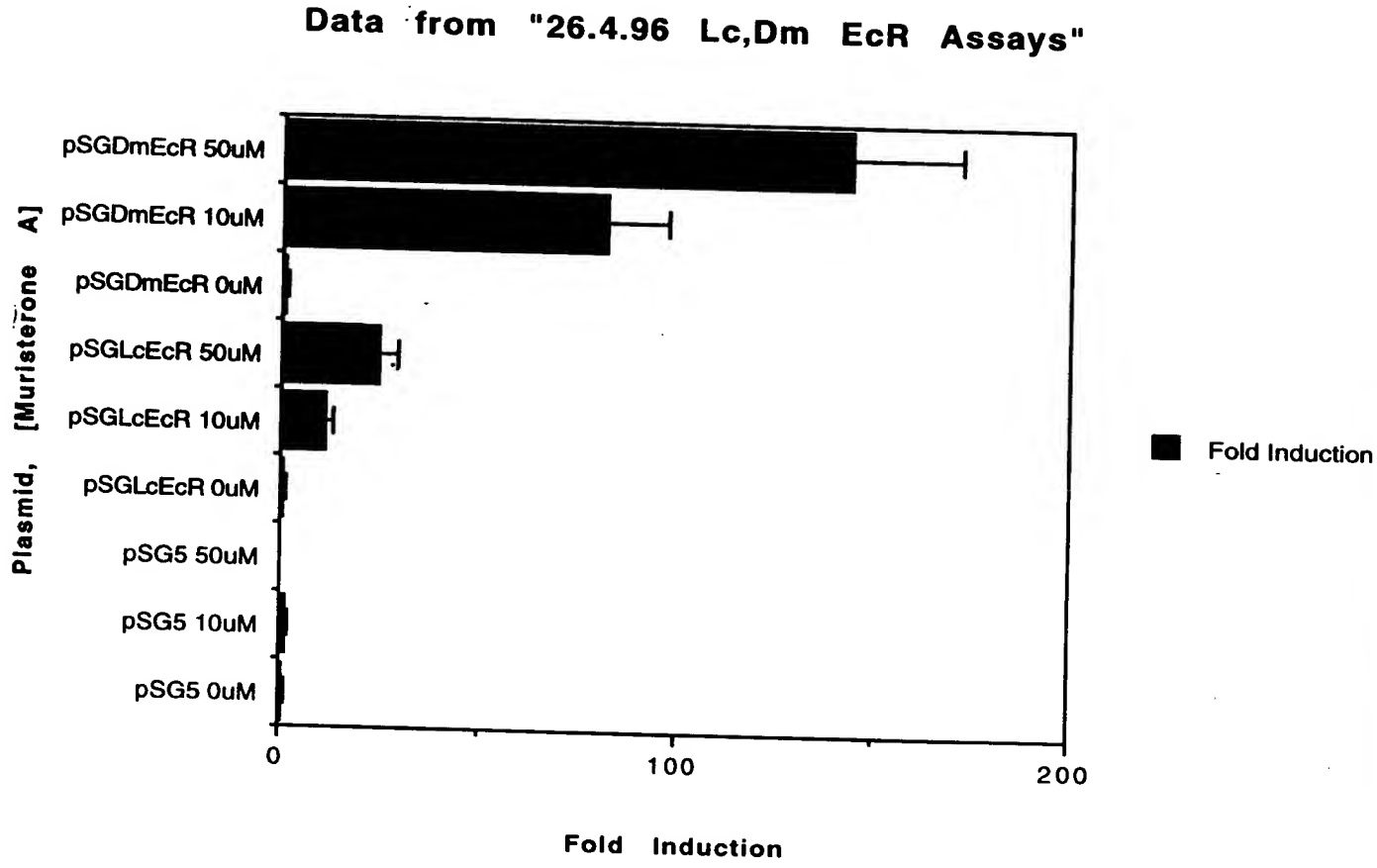

Data from "26.4.96 Lc,Dm EcR Assays"
pSGDmEcR 50uM
pSGDmEcR 10uM
pSGDmEcR 0uM
pSGLcEcR 50uM
pSGLcEcR 10uM
pSGLcEcR 0uM
pSG5 50uM
pSG5 10uM
pSG5 0uM
Plasmid, [Muristerone A]
0
100
200
Fold Induction
Fold Induction

FIGURE 7:

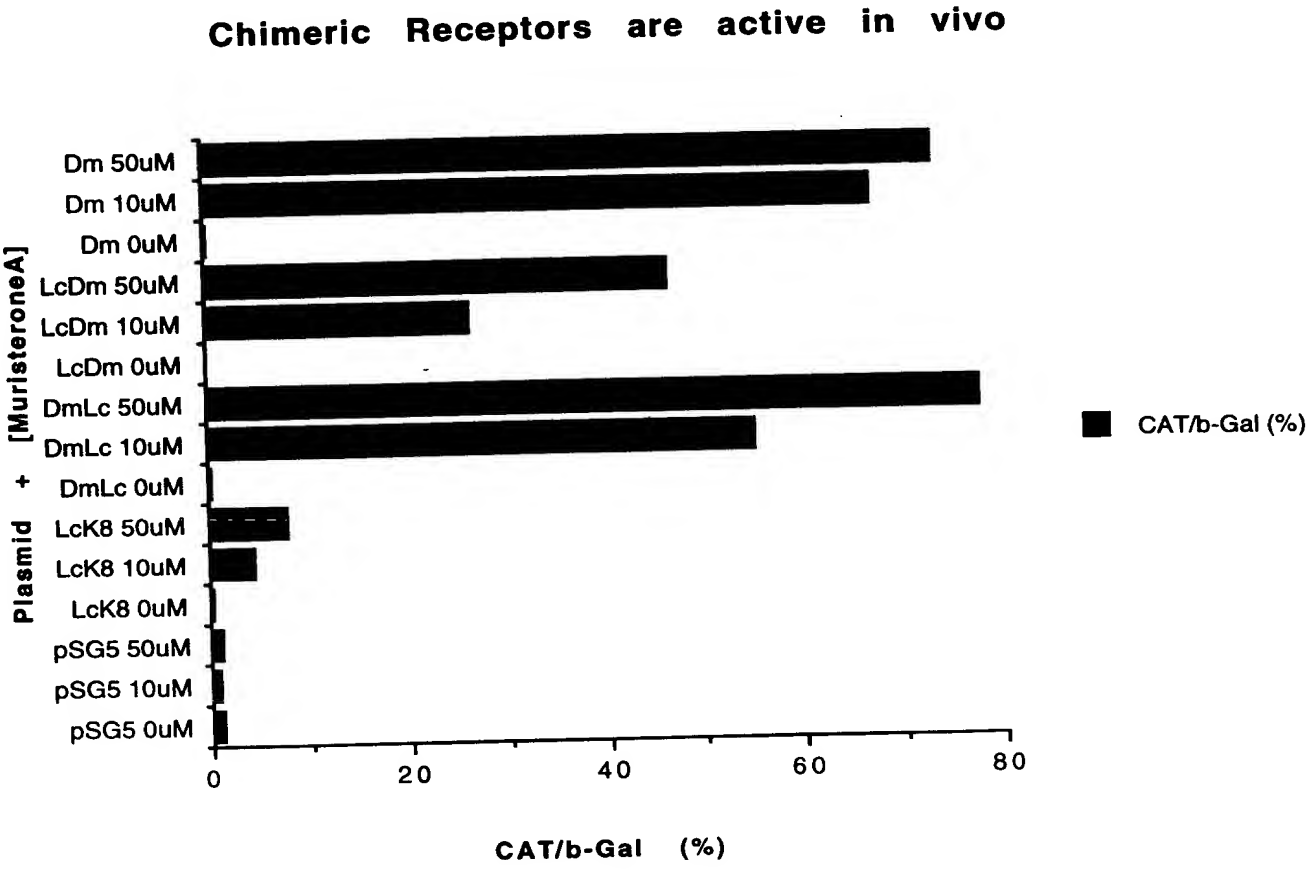
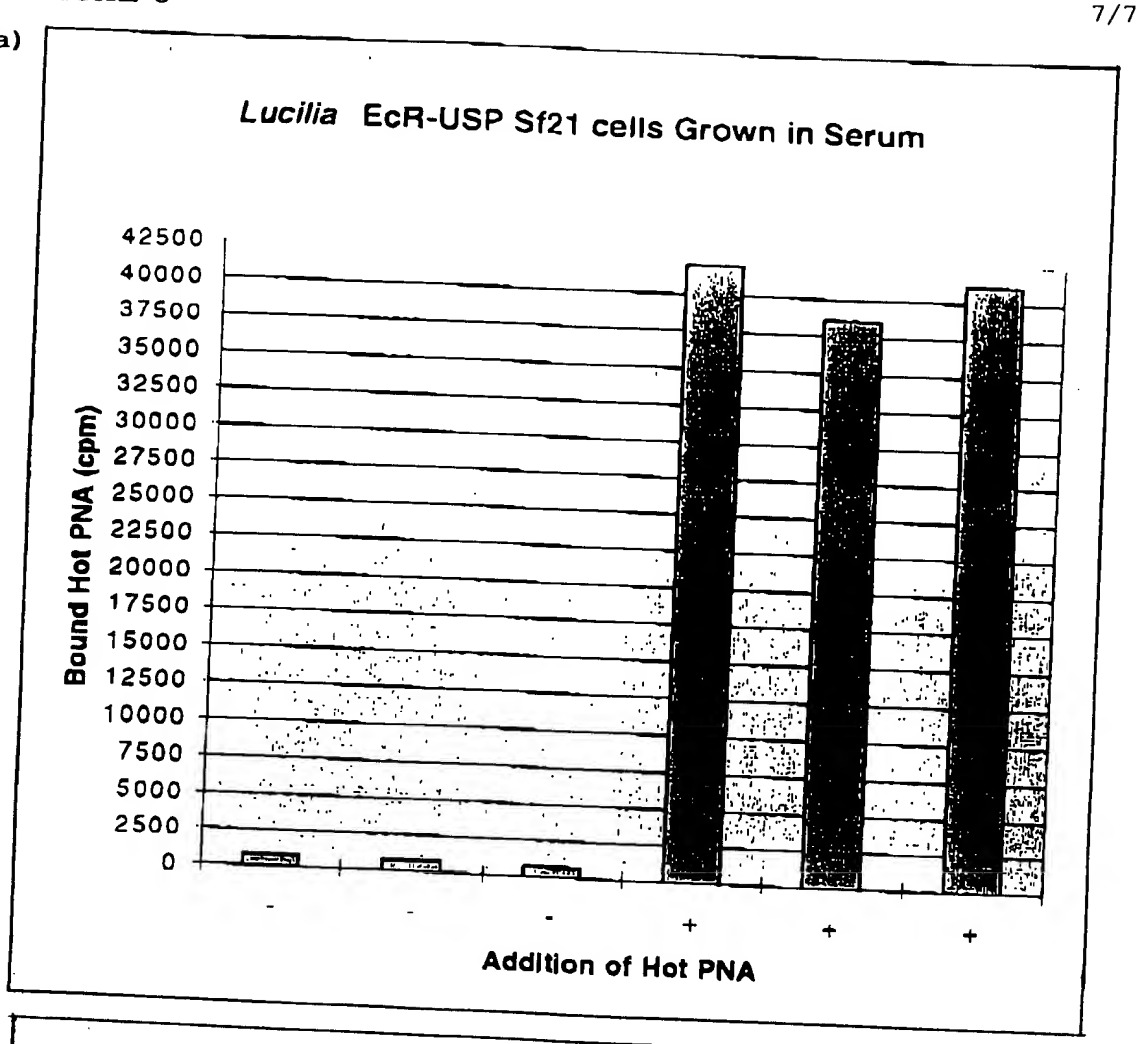
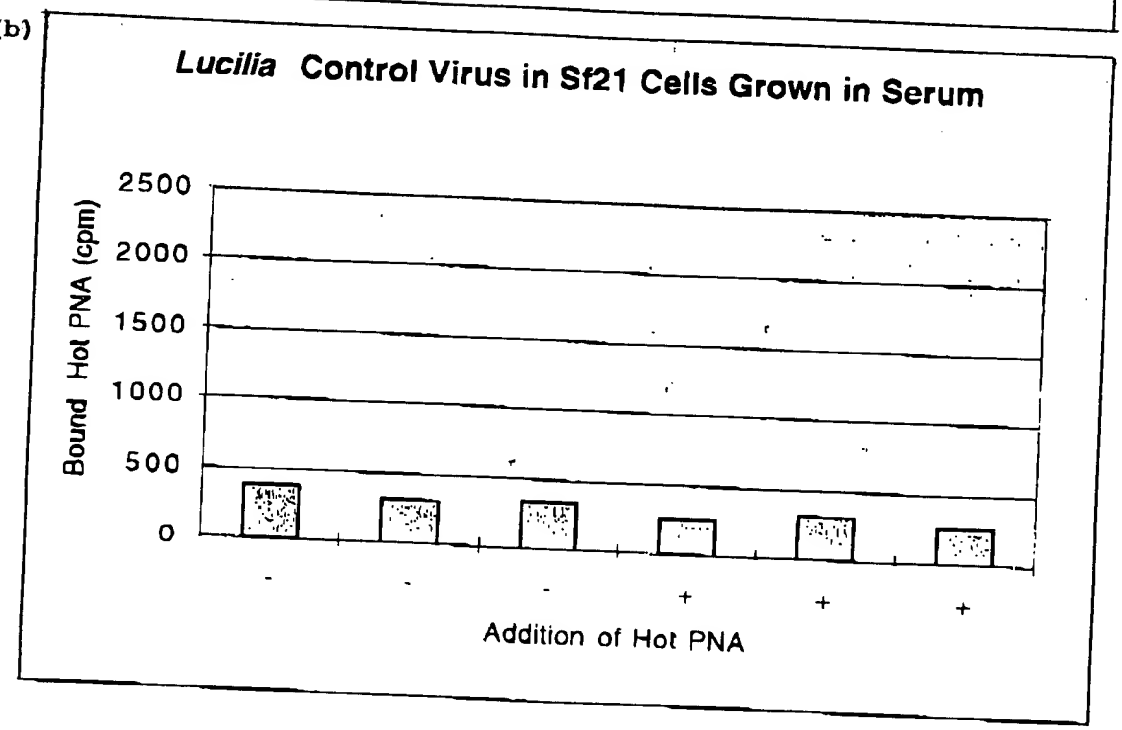

Chimeric Receptors are active in vivo
Plasmid + [MuristeroneA]
Dm 50uM
Dm 10uM
Dm 0uM
LcDm 50uM
LcDm 10uM
LcDm 0uM
DmLc 50uM
DmLc 10uM
DmLc 0uM
LcK8 50uM
LcK8 10uM
LcK8 0uM
pSG5 50uM
pSG5 10uM
pSG5 0uM
0
20
40
60
80
CAT/b-Gal (%)
CAT/b-Gal (%)

FIGURE 8

(a)


Lucilia EcR-USP Sf21 cells Grown in Serum
Bound Hot PNA (cpm)
42500
40000
37500
35000
32500
30000
27500
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
- - - + + +
Addition of Hot PNA

(b)


Lucilia Control Virus in Sf21 Cells Grown in Serum
Bound Hot PNA (cpm)
2500
2000
1500
1000
500
0
- - - + + +
Addition of Hot PNA